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**Compilation of 1987 Annual Reports
of the Navy ELF Communications System
Ecological Monitoring Program**

Volume 2 of 3 Volumes:
TABS D - G

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Technical Report E06595-2
Contract No. N00039-88-C-0065
August 1988

Prepared for:

Communications Systems Project Office
Space and Naval Warfare Systems Command
Washington, D.C. 20363-5100

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Band, R. N.
- E. Soil and Litter Arthropoda and Earthworm Studies
Michigan State University
Snider, R. J.; Snider, R. M.
- F. Biological Studies on Pollinating Insects: Megachilid Bees
Michigan State University
Strickler, K.; Scriber, J. M.
- G. Small Vertebrates: Small Mammals and Nesting Birds
Michigan State University
Beaver, D. L.; Asher, J. H.; Hill, R. W.



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FOREWORD

The U.S. Navy is conducting a long-term program to monitor for possible effects from the operation of its Extremely Low Frequency (ELF) Communications System to resident biota and their ecological relationships. The program is being implemented by IIT Research Institute (IITRI) under contract to the Space and Naval Warfare Systems Command (SPAWAR). IITRI provides engineering support and coordinates the efforts of investigators. Monitoring projects are being carried out through subcontract arrangements between IITRI and study teams at several universities.

This is the sixth compilation of annual reports prepared by university study teams. Each report chronicles the data collection and data analysis activities for a monitoring project during 1987. As in the past, each report has been reviewed by four or more scientific peers. Investigators have considered and addressed reviewer critiques prior to providing their report for printing. Reports have been printed from original copies without change or editing by either IITRI or SPAWAR.

Reports other than this compilation document electromagnetic exposures at study sites and summarize the annual progress of the program. These reports have been prepared on an annual basis since the inception of the program in 1982. All have been provided to the National Technical Information Service for unlimited distribution. The results of monitoring studies have also been presented at scientific meetings and as articles in peer reviewed, scientific journals.

ELF ECOLOGICAL MONITORING PROGRAM
INDEX OF 1987 ANNUAL REPORTS

- A. Herbaceous Plant Cover and Tree Studies
Michigan Technological University
Becker, C. T.; Brooks, R. H.; Bruhn, J. N.; Cattelino, P. J.;
Gale, M. R.; Holmes, M. J.; Jurgensen, M. F.; Liechty, H. O.;
Moore, J. A.; Mroz, G. D.; Reed, D. D.; Reed, E. J.; Richter, D. L.;
Zhang, Y. F.
- B. Litter Decomposition and Microflora
Michigan Technological University
Bagley, S. T.; Bruhn, J. N.; Pickens, J. B.
- C. The Effects of Exposing the Slime Mold Physarum polycephalum
to Electromagnetic Fields
University of Wisconsin-Parkside
Goodman, E. M.; Greenebaum, B.
- D. Soil Amoeba
Michigan State University
Band, R. N.
- E. Soil and Litter Arthropoda and Earthworm Studies
Michigan State University
Snider, R. J.; Snider, R. M.
- F. Biological Studies on Pollinating Insects: Megachilid Bees
Michigan State University
Strickler, K.; Scriber, J. M.
- G. Small Vertebrates: Small Mammals and Nesting Birds
Michigan State University
Beaver, D. L.; Asher, J. H.; Hill, R. W.
- H. Aquatic Ecosystems
Michigan State University
Burton, T. M.; Stout, R. J.; Taylor, W. W.; Oemke, M. P.;
Whelan, G.
- I. Wetland Studies
University of Wisconsin-Milwaukee
Stearns, F.; Guntenspergen, G.; Keough, J.; Wikum, D.
- J. Bird Species and Communities
University of Minnesota-Duluth
Hanowski, J. M.; Niemi, G. J.; Blake, J. G.

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Michigan State University
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3. Table of Contents:

1. Cover Page.....	i
2. Frontispage.....	iii
3. Table of Contents.....	v
4. Abstract.....	vi
5. Summary.....	vii
6. Progress Report.....	1
a. Objectives.....	1
b. Work Plan.....	2
c. Experimental.....	9
7. Peer Reviewers.....	15
8. Literature cited.....	16
9. Tables and Figures.....	18

4. Abstract:

The most outstanding aspect of the 1987 field season was the continued effect of the 1986 drought. It suppressed population growth of soil amoebae in 1986 and decreased genetic heterogeneity of one soil amoeba species. Population growth was also suppressed to a lesser extent in 1987.

Antenna, ground and control sites, used in previous seasons were continued. The sites have been characterized as to electromagnetic background (by IITRI personnel), physical and chemical properties, and biological characteristics.

Studies of soil amoebae in 1983 were designed to provide sufficient data to determine sample sizes and methods of statistical analysis suitable for comparing control and experimental sites. These were utilized in the 1984, 1985, 1986 and 1987 field seasons. Fluctuations in total amoeba number were observed in both the 1984 and 1985 growing seasons at control, antenna and ground study sites. The 1986 drought suppressed significant growth of soil amoebae. At a lower level, population growth fluctuations were noted again in 1987.

The genetic diversity within a species of soil amoeba was determined by isoenzyme analysis of clones isolated from control, antenna and ground sites. To test the effect of ELF electromagnetic radiation on the growth of amoebae culture chambers were designed and constructed by IITRI personnel. These were used at the Wisconsin Transmitter Facility in 1986.

5. Summary:

Plot selection and characterization: Site selection has been completed. Soil chemistry was performed on all sites in 1987, with a statistical comparison of sites. As in 1986, there were some differences between sites in terms of soil chemistry. Possibly this was due to the dry season in 1986 and early 1987.

Species and strain characterization: Acanthamoeba polyphaga was used to test for strain heterogeneity within the sites. Isoenzyme analysis was chosen as the technique to detect strain differences. Sufficient heterogeneity was observed between clonal isolates to make this a useful technique to detect possible effects by ELF electromagnetic radiation on heterogeneity within a species. A paper on this was published in the J. Protozoology. Restriction fragment analysis of mitochondrial DNA from Naegleria species has been accepted for publication.

Population size: The total population of amoebae increased during the 1984 and 1985 growing seasons. Toward the end of both growing seasons, the total population of amoebae decreased. Population sizes were the same at control, antenna and ground sites. The ratio between vegetative amoebae and dormant cysts was labile both within and between sites. The biological basis for population fluctuations are being investigated.

Probably due to the drought, little growth was observed in the 1986 season although population size did not differ statistically between study sites. Although population numbers in 1987 did not reach the 1984/85 levels, there was a fluctuation in the population similar to those years.

Growth and feeding activity: In the 1984 season, studies on growth in culture chambers by A. polyphaga demonstrated no significant difference in growth between sites. This was done to test the experimental and statistical means for examining growth "under the wire" and to test the culture chamber design. By the 1985 season, the electrical circuits associated with the culture chamber was designed and constructed by IITRI personnel. An attempt was made to test the unit at the Wisconsin Transmitter Facility, but it was not satisfactory because the antenna was not running long enough to do the experiments at those times chosen to work. In 1986 it was possible to do a growth experiment while the transmitter was running. The brief experiment failed to reveal differences in growth between control and experimental culture chambers, but it did provide an opportunity to test the system. In 1987 growth experiments were conducted at the Michigan site. These revealed technical problems that have been solved. Feeding efficiency methods were developed as well.

Ambient monitoring: Soil temperature and moisture were monitored continuously during the 1984 and 1985 field seasons (June through September). In both seasons the ambient data did not correlate to changes in amoeba population. The moisture content of soil in 1986 and 1987 was lower. This and total Spring rainfall correlated to small populations of amoebae in soil.

1.

6. Progress report:

OBJECTIVES: The project objective is to determine effects of ELF radiation on amoebae in soil. The sites chosen for this study are adjacent to the Michigan ELF transmitter.

For the 1987 field season, as was true for the previous seasons, the primary objective was to demonstrate that the control, antenna and ground wire study sites were biologically similar in regards to soil amoebae. In addition a base line was accumulated for comparison with future data, especially that obtained once the antenna is fully operational.

WORK PLAN ELEMENTS:

#0. Plot selection and characterization.

Synopsis: Site selection is complete. Statistical analysis of soil chemistry shows variability between sites. This may have been due to the exceptionally dry season in 1986 and a dry Spring in 1987.

#1. Species and strain characterization.

Synopsis: using morphological and physiological markers, identify species and strains of soil amoebae from the study areas so that possible changes in the population due to ELF can be detected.

Specifics: Species of soil amoebae present at the study sites are isolated from soil enrichment plates. In this way, clonal isolates of A. polyphaga were obtained from control, antenna and ground sites for isoenzyme analysis. Soil amoebae are asexual organisms, reproducing without apparent sexual, genetic recombination. However, isoenzyme analysis reveals significant heterogeneity between clonal isolates. The isoenzyme patterns are the same as those observed for sexually reproducing, diploid organisms. For this reason, the analytical and statistical techniques developed for isoenzyme

analyses of higher organisms is used in the present study. There is a precedence for this approach, it has been used recently to examine laboratory isolates of Naegleria species (i.e. Pernin et al. 1985). The "genetic distance" between clonal isolates and between sites were determined by Nei's method (Nei, 1972), a widely used mathematical expression of the relationships between related organisms. This approach has been used to study inter and intra-species relationships (e.g. Avise et al., 1975). We have published a paper on the genetic heterogeneity observed at the study sites (Jacobson & Band, 1987).

It was suggested in the past that I use species diversity/richness indices. This is not feasible, as noted in the 1984 annual report. Not enough personnel are available to do the work necessary for this analysis. Since all of the species observed do not appear on similar dilution plates (see the next work plan element), many more plates would have to be set-up than is required for counting amoebae. As pointed out by one of the reviewers, possible changes in species diversity would be preceded by changes in genetic heterogeneity within the species.

We have a paper accepted for publication on mtDNA and evolutionary relationships within the genus Naegleria. A mtDNA analysis of A. polyphaga clonal isolates may be needed to demonstrate species relationships.

#2. Population size and activity.

Synopsis: determine population size of amoebae in soil and the ratio of vegetative to dormant amoebae over the growing season. This is a productivity measure which could be affected by ELF radiation, it could also be a reflection of changes in the microbial food organisms due to ELF radiation.

Specifics: an established soil dilution counting technique is used (Singh, 1946 as modified by Darbyshire et al., 1974). In order to count vegetative amoebae and cysts, samples are first divided in half, one-half is used to count total cysts and vegetative amoebae while the other half is treated to kill amoebae so that only cysts are counted. Differential counts are used to calculate by subtraction the total vegetative amoeba count. In the 1983 season I found that 8 random samples, subdivided into organic and mineral horizons (i.e. 8 samples per horizon), provided statistically significant data. Two-way analysis of variance was used to detect differences in total amoeba and cyst count between control, antenna and ground sites for each horizon in 1987. Table 4B gives the error (i.e. among) degrees of freedom as 21. Direct counts of amoebae in soil, as is done with freshwater organisms (e.g. Wright & Coffin, 1984), is not possible. Microbes adhere to soil and sonication of a soil slurry to release them might make quantitative recovery of bacteria by subsequent density flotation possible, but amoebae would be destroyed.

5.

#3. Growth and feeding activity.

Synopsis. Determine the in situ growth and feeding activity of amoebae in soil submersible culture vessels. This will provide data on growth rate, feeding activity and mean generation time (i.e. the cell cycle between nuclear mitoses).

Rationale. The approach utilizes a known amoeba species previously isolated from the study site, Acanthamoeba polyphaga and characterized as part of the isoenzyme study. Direct counts of amoebae are made with a microscope to determine increase in number of organisms and nuclei over time. A log transform of these data provides a straight line plot which can be quantified by regression analysis. Statistically significant differences between slopes can be detected with confidence limits of the line, a version of the t-test. This approach will be used to determine growth rate and thus mean generation time. Mean generation time is comparable to the cell cycle measurement of time between mitoses of Physarum. Cropping activity will be determined by counting the number of bacteria eaten during amoeba growth. Culture chambers, containing electrodes to eventually use in conjunction with ELF induced currents, were designed with the help of IITRI personnel. These electrodes will be connected with electrodes buried in soil adjacent to the culture chambers, to produce the necessary voltage drop from the current in the soil, induced by ELF radiation.

In the 1984 field season, it was demonstrated that counts from chambers buried at the research sites yielded growth rates that were statistically the same. For the 1985 season, IITRI personnel designed and constructed the electrical components to interface between the soil electrodes and the culture chambers. It was not possible to test these under the Wisconsin Transmitter antenna in 1985 because it was only periodically operational. We were able to perform growth experiments in 1986 at the Wisconsin transmitter. In 1987, long-term incubations were attempted in Michigan to detect equipment defects and procedural problems. The electrical interface between the culture chamber, containing electrodes to pass a current through the culture saline from soil electrodes, is necessary because it is not possible to mimic electrical properties of soil in a physiological saline. The soil electrodes consist of copper pipe, 4" diameter and 3' long. Soil water is a dilute saline, suitable for amoeba growth, but it is not a continuous phase across a significant space. Therefore soil exhibits higher resistance than would be the case for soil water. In the case of physiological saline in the culture chambers, the resistance across the electrodes is much lower than a comparable distance in soil. If growth in soil were used, it would mimic normal soil properties but cell counting procedures would lose a significant degree of accuracy since enrichment counting procedures would be required. Therefore, two different culture chamber arrangements are needed, one to mimic the voltage induced in soil and the other to mimic the current.

#4. Ambient monitoring.

Synopsis. Soil temperature and moisture are monitored. Both measures are useful for general trends but failed to correlate to changes in amoeba populations in previous field seasons. The dry spell during all of the 1986 growing season did suppress amoeba populations, and this did extend into the 1987 season to a lesser extent.

#5. Data analysis.

Synopsis. Statistical analyses mentioned earlier are summarized here. For amoeba counts in soil, by soil dilution procedures, a one-way analysis of variance with 8 replicates per cell was adequate. One-way analysis of variance was used for soil counts (Table 4B) and soil moisture (Table 5) because it is not possible to compare soil horizons or sampling dates. The lower, mineral horizon is roughly twice as dense as the upper, organic horizon so that number of amoebae/g soil and soil moisture differs between horizons. Likewise differences between sampling dates preclude similar comparisons. Growth measurements in culture chambers were analyzed with regression lines, comparing slopes with confidence intervals (a t-test). Other statistical comparisons (e.g. soil chemistry, soil pH, etc.) are done by analysis of variance. For isoenzyme determinations the mainframe computer is needed to do the calculations by Nei's method (Nei, 1972).

8.

SCHEDULE OF WORK ELEMENTS (Nov. 1 to Oct 31 each year)

[illegible]

EXPERIMENTAL

Methods and results will be presented in reference to the Work Plan, given above.

#0. Plot selection and characterization. Site selection is now complete.

Table 1 shows the chemical properties of the organic and mineral horizons for the control, antenna and ground wire sites, with replicates. As in the 1986 season, differences exist between sites. This might be attributable to the 1986 dry season which extended into early Spring of 1987. A reviewer of this report pointed out that soil Ca at the antenna site, organic layer increased by 50% from 1983 to 1987. Again, the organic nitrogen content of soil in the organic horizon from both the antenna and ground sites decreased by approx. 70% from 1983 to 1987. These trends will be followed in subsequent years. Table 2 demonstrates some significant differences between sites and sampling dates. Table 3 demonstrates the slightly acidic nature of the soil in a northern hardwood forest, without significant differences between horizons or sampling dates, but some differences between sites (at the 5% significance level).

#1. Species and strain characterization. Species of soil amoebae present at the study sites were isolated from soil enrichment plates. So far no species differences have been noted between sites; species composition was the same as in previous field seasons. Species included Acanthamoeba castellanii, A. polyphaga, A. astronyxis (small strain), Hartmannella sp., Rosculus sp., Naegleria gruberi, Vahlkampfia sp., and Mayorella sp. For the isoenzyme analysis, I have chosen A. polyphaga. A. polyphaga is no more common in soil isolates than other amoebae but its cyst is very distinctive which makes it easy to pick out from soil dilution, enrichment plates (see #2 below). Isoenzyme analyses of 10 clone isolates from each of the 3 study sites were done in the 1986 season and reported here (Table 7); there were no significant differences between sites. I am using the same isoenzymes as those used by the American Type Culture Collection (Daggett & Nerad, 1983). Included in the isoenzymes are some that have been used by others, e.g. Pernin et al., 1985. The enzymes used were: malic enzyme, isocitrate dehydrogenase, leucine amino peptidase, phosphoglucomutase, hexokinase, lactate dehydrogenase, 6-phosphogluconate dehydrogenase, arginine amino peptidase, glutamate dehydrogenase, L-threonine dehydrogenase, beta-hydroxybutyrate dehydrogenase, butyryl esterase 2, propionyl esterase, acetyl esterase. When I did the 1985 isoenzyme study, I was only able to do a few isoenzymes; since then I have improved the homogenization method so that it is possible to do many more isoenzymes.

In retrospect, the original method I used was designed by others for pure cultures (i.e. axenic cultures) as opposed to my objective of using bacterized cultures to avoid genetic selection. Isoenzyme patterns of A. polyphaga show differences between isolates consistent with a diploid organism. Nei's method for measuring genetic difference (Nei, 1972) was used in this study. The antenna site analysis was done with 137 alleles; the ground site was done with 143 alleles; the control site was done with 144 alleles. A paper on this work has been published (Jacobson & Band, 1987). The 1987 season data is still being analyzed. Data completed for the 1986 season indicated a significant drop in genetic diversity at all sites (Table 7). This genetic bottleneck may have been due to the drought which would imply that a significant part of the amoeba population died, leaving survivors with a narrow genetic variability. This supports the sensitivity of isoenzymes to physiological stress in the natural population. If ELF radiation introduces stress, it should be reflected in a reduction of isoenzyme diversity as well. Data analysis for the 1987 season may be available for the final version of the annual report.

#2. Population size and activity. From the 1983 field data, the number of replicate samples used to determine population size at each study site (i.e. 10) was more than adequate since the coefficient of variation was <10% of the mean.

For a single site, 10 samples, 1 date and 9 D.F., a significant difference at the 90% probability level would be $1.4 \times \text{S.D.}$ (from a power curve); for 8 samples per site this would drop a little to 1.5 to $1.6 \times \text{S.D.}$ For the 1984, 1985 and 1986 field seasons I chose to use 8 replicates per site since there was little loss in power between 10 and 8 replicate samples per site. Darbyshire's 96 multiwell adaptation of Sing's soil dilution method (Darbyshire et al. 1974) was used. The results of the 1987 season differed from 1986 in exhibiting larger amoeba populations. However, the control, antenna and ground wire sites did not have the same number of amoebae/g soil in the organic and mineral horizons for the 6/16 and 7/21 counts (Table 4, 4B and Figs 1 to 5, 15, 16, & 17). Data on the distribution of amoebae between the cyst and vegetative stages indicates that a proportion of amoebae are in the dormant state for much of the season and vegetative amoeba distribution does differ between sites (Table 4A, 4B and Figs. 1 to 3 and 6 to 9). Specifically, Table 4 gives total counts of vegetative amoebae and cysts while Table 4A gives counts of cysts alone, thus the mathematical difference gives the number of vegetative amoebae present in a sample. Figs. 1, 2 and 3 represent Tables 4 and 4A in showing total counts and cyst counts by horizon and site at various sampling dates. Fig. 4 and 5 compare total counts by horizon.

The mathematically calculated number of vegetative amoebae is given in Figs. 6 & 7, while the percent vegetative amoebae is given in Figs. 8 & 9. The 1987 season was not as dry as the 1986 season, according to the Climatological Data publications of the Climatic Data Center, NOAA, although April was well below normal in rainfall (Fig. 14). This may be responsible for the slow recovery from the 1986 drought population. The general population fluctuation observed in past years was evident in 1987, while in 1986 it was hardly detectable. Soil moisture in itself probably did not directly limit growth of amoebae. Nutrient input to the soil was probably limited by the dry season which in turn produced a small microbial population for the amoebae to feed on.

#3. Growth and feeding activity. In 1987 growth experiments were attempted at the experimental sites in Michigan and a method was developed to measure bacterial feeding rates by amoebae. Attempts to do growth experiments in soil submersible culture vessels over several days revealed that the solder joint between the electrical connector and the electrode corroded; this led to a variety of spurious electrical measurements. Silicone sealing the electrode into the ends of the culture vessel only delayed corrosion. Ultimately this was solved by coating the solder joint with polyurethane glue rather than attempting to seal the electrode into the culture vessel.

I also found that it was not possible to sample and count at each site on the same day.

In order to do parallel experiments at the three sites, it is necessary to fix samples with glutaraldehyde and do the counts in the lab.

Table 6 and Fig. 16 to 19 are amoeba growth experiments coupled with bacterial feeding counts done in the laboratory. The slope of the amoeba's growth and consumption of bacteria was the same at 2 densities of amoebae and bacteria (Table 6, Fig. 16 & 17). All Y axes (no. of organisms) were log plots. The bacterial quantity for trial #1 was 2 mg dry wgt./ml and for trial 2 it was 1 mg/ml. The feeding rate/ 2hr. intervals was proportional to bacterial density over the growth curve (Fig. 18 & 19). Bacterial suspensions incubated without amoebae did not change in number over the experiment.

#4. Ambient monitoring. Table 5 (and Figs. 12 & 13) gives the mean % (w/w) moisture for individual measurements, taken when the soil was sampled, for each set of 8 replicate samples per horizon/site/date. During the growing season (i.e. June, July and August) the soil was drier than in 1984 and 1985, and roughly comparable to 1986.

Soil temperature recordings for the season (Fig. 15) were somewhat warmer than for previous seasons. It will be interesting to compare this with the 1988 season to see if this is a long term trend. Since NOAA air temperatures did not indicate above average temperatures for 1987, I assume the soil temperature differences were due to foliar shading differences.

7. Peer reviewers:

I plan to use the following individuals as peer reviewers:

- a. Prof. Thomas J. Byers
Department of Microbiology
Ohio State University
- b. Prof. Fredrick L. Schuster
Department of Biology
Brooklyn College

LITERATURE CITED

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TABLE 1. SOIL CHEMISTRY:*

		SITE/ HORIZON**					
ELEM.	DATE***	CO	AO	GO	CM	AM	GM
P	1	54.47	32,37	34,34	74.71	14,15	29,30
	2	40,43	30,31	36,37	69,69	14,14	18,19
K	1	96,100	136,104	112,116	36,36	36,36	32,32
	2	93,80	84,106	89,97	35,35	31,31	31,31
Ca	1	1960,1760	2600,2280	2520,2480	696,731	920,765	765,731
	2	2300,1950	2484,2863	2400,2400	572,610	840,840	800,762
Mg	1	109,109	157,149	153,160	54.54	76,67	67.67
	2	129,115	139,164	153,153	49,45	87,91	71.62
NO ₃	1	21.3,20.3	24.1,20	25.4,23	5.8,6.7	10.4,7.2	8.6,11.8
	2	29.1,23.7	38.7,34.2	33.2,29.8	6.9,6.7	33.2,21.7	23.37
%Org.N.1		5.1,5.7	4.1,4.2	5.4,5.0	1.0,1.0	1.3,1.2	1.3,1.1
	2	6.7,5.7	5.1,6.5	5.5,5.8	1.0,1.0	1.4,1.24	1.2,1.3

* Performed by Michigan State University Soil Testing Laboratory. data expressed as ppm except for %org.N.

** SITE: C. control; A, antenna; G, ground.
HORIZON: O. organic; M. mineral

*** Data was obtained June 1 and Aug. 17, 1987, each of which were taken from 20 random samples.

TABLE 2. SOIL CHEMISTRY 2X ANOVA: Two-way analysis of variance between sites /dates:

ELEMENT		ORGANIC			MINERAL		
		D.F.	M.S.	F		M.S.	F
P	Site	2	203.58	28.74 **		3683.08	2326.2 **
	Date	1	36.75	5.19 NS		80.08	50.579 **
	Interact.	2	33.25	4.69 NS		28.58	18.503 **
	Error	6	7.08	----		1.58	----
K	Site	2	250.08	1.69 NS		144.08	2.06 NS
	Date	1	1102.08	7.46 *		70.08	1.004 NS
	Interact.	2	48.08	0.33 *		70.08	1.004 NS
	Error	6	147.75	----		69.75	----
Ca	Site	2	430836.58	13.96 **		36141.08	14.804 **
	Date	1	82834.09	2.69 NS		2821.33	1.1557 NS
	Interact.	2	57456.59	1.8627 NS		6640.08	2.72 NS
	Error	6	30845.07	----		2441.16	----
Mg	Site	2	1931.58	24.82 **		887.583	54.902 **
	Date	1	21.33	0.274 NS		33.33	2.0619 NS
	Interact.	2	81.08	1.041 NS		161.08	9.964 *
	Error	6	77.83	----		16.166	----
NO ₃	Site	2	72.254	4.92 NS		215.16	7.38 *
	Date	1	34.6	1.59 **		506.99	17.40 **
	Interact.	2	248.42	11.40 NS		116.58	4.00 NS
	Error	6	21.79	----		29.13	----
%Org N	Site	2	0.683	2.288 NS		0.09029	12.65 **
	Date	1	2.803	9.397 *		0.00479	0.673 NS
	Interact.	2	0.3808	1.276 NS		0.001299	0.1822 NS
	Error	6	0.2983	----		0.007133	----

* = 5% significance level

** = 1% significance level

TABLE 3. SOIL pH:

DATE	SITE	HORIZON	MEAN pH \pm S.D. (n=10)
3JUN87	Control	Organic	6.2 \pm 0.45
		Mineral	6.5 \pm 0.37
	Antenna	Organic	6.64 \pm 0.43
		Mineral	6.50 \pm 0.34
	Ground	Organic	6.66 \pm 0.54
		Mineral	6.5 \pm 0.49
6JULY87	Control	Organic	6.2 \pm 0.37
		Mineral	6.4 \pm 0.36
	Antenna	Organic	6.7 \pm 0.42
		Mineral	7 \pm 0.23
	Ground	Organic	6.7 \pm 0.54
		Mineral	6.4 \pm 0.49
17AUG87	Control	Organic	6.1 \pm 0.69
		Mineral	6.3 \pm 0.52
	Antenna	Organic	6.7 \pm 0.29
		Mineral	6.6 \pm 0.26
	Ground	Organic	6.6 \pm 0.39
		Mineral	6.6 \pm 0.45

Three-way ANOVA:

F-TESTS

	D.F.	M.S.	Test	F	
#1. Site	2	2.10489	1	11.8228	*
#2. Horizon	1	0.01800918	2	0.1012	NS
#3. Date	2	0.0895586	3	0.5030	NS
#4. Site X Horizon	2	0.480608	4	2.6995	NS
#5. Site X Date	4	0.12855	5	0.7220	NS
#6. Horizon X Date	2	0.044659	6	0.2508	NS
#7. Site X Horizon X Date	4	0.14483737	7	0.8135	NS
Error	162	0.178037019			

Table 4. Total counts from 8 samples per horizon/site:

SITE	HORIZON	DATE	MEAN #/g soil [*] ± S.E.	MEAN ^{**} (#/g soil)
Control	Organic	6/16	8.0644 ± 0.1537	3454
		7/21	9.8203 ± 0.1552	19990
		8/25	9.8054 ± 0.2530	22599
		9/9	8.1987 ± 0.1561	3934
		10/16	7.8569 ± 0.2724	3345
	Mineral	6/16	7.4150 ± 0.1295	1756
		7/21	9.2536 ± 0.1080	10884
		8/25	9.3049 ± 0.2862	15281
		9/9	6.7134 ± 0.1578	905
		10/16	6.3775 ± 0.1199	619
Antenna	Organic	6/16	9.1587 ± 0.1373	10121
		7/21	10.7984 ± 0.1362	52262
		8/25	10.3643 ± 0.2000	37430
		9/9	7.7602 ± 0.1753	2572
		10/16	7.8025 ± 0.3391	2822
	Mineral	6/16	8.4552 ± 0.0992	4869
		7/21	9.3509 ± 0.1412	10622
		8/25	9.6791 ± 0.2509	19225
		9/9	6.6476 ± 0.1054	803
		10/16	6.1453 ± 0.0887	481
Ground	Organic	6/16	8.5523 ± 0.1552	5724
		7/21	9.6968 ± 0.2628	20703
		8/25	10.4918 ± 0.2000	40689
		9/9	8.2677 ± 0.1931	4422
		10/16	7.3273 ± 0.1259	1607
	Mineral	6/16	7.4210 ± 0.1080	1720
		7/21	8.2307 ± 0.0646	3811
		8/25	9.9369 ± 0.1335	22048
		9/9	6.8478 ± 0.1588	1020
		10/16	6.3079 ± 0.0607	556

* Mean expressed as the natural log of amoeba number, used to calculate analysis of variance (Table 4B).

** The mean calculated from log transformed data and the mean calculated from the original arithmetic data cannot be interchanged. See page 22.

Table 4 footnotes continued:

I had assumed computer roundoff was the problem in converting between log and arithmetic means, this is not the case. The following calculations, using the 1987 field counts, illustrate this:

I. Control site, organic horizon, total count for Oct. 16, 1987.

	#/g dry soil	ln transformed #/g dry soil
	5101	8.5371918
	1963	7.58222919
	1654	7.4109518
	982	6.8895913
	1114	7.0157124
	4959	8.4335941
	8564	9.05532265
	2783	7.931284762
MEANS:	3345	7.856984753
	ln transform: 8.115221973	

II. Antenna site, mineral horizon, total count for June 16, 1987.

	5983	8.696677393
	2991	8.003363059
	4622	8.438582791
	7786	8.960082528
	4233	8.350666241
	4869	8.490643856
	4233	8.350666241
	4233	8.350666241
MEANS	4896	8.455168544
	ln transform: 8.490643856	

Table 4A. Cyst counts from 8 samples per horizon/site:

SITE	HORIZON	DATE	MEAN #/g soil* ± S.E.	MEAN (#/g soil)
Control	Organic	6/16	7.8712 ± 0.2162	3216
		7/21	9.7821 ± 0.2189	20817
		8/25	8.9579 ± 0.1908	8732
		9/9	7.9356 ± 0.1602	3041
		10/16	8.1632 ± 0.2191	3614
	Mineral	6/16	6.7330 ± 0.0855	863
		7/21	8.2906 ± 0.0874	4091
		8/25	6.9413 ± 0.1761	1156
		9/9	6.6486 ± 0.1124	807
		10/16	6.5518 ± 0.1198	738
Antenna	Organic	6/16	8.3050 ± 0.1421	4292
		7/21	9.7842 ± 0.1056	18526
		8/25	8.8044 ± 0.2921	8917
		9/9	8.1823 ± 0.1543	3883
		10/16	8.0831 ± 0.1721	3539
	Mineral	6/16	6.6174 ± 0.0694	761
		7/21	8.1284 ± 0.1995	3828
		8/25	7.0437 ± 0.1486	1243
		9/9	6.8239 ± 0.1301	976
		10/16	6.0365 ± 0.0567	423
Ground	Organic	6/16	8.3179 ± 0.2191	4564
		7/21	9.7387 ± 0.1379	18026
		8/25	9.3108 ± 0.2649	13892
		9/9	8.1186 ± 0.2428	4116
		10/16	8.0597 ± 0.1910	3118
	Mineral	6/16	6.5530 ± 0.0965	723
		7/21	8.4373 ± 0.2051	5286
		8/25	7.1321 ± 0.1770	1391
		9/9	6.7934 ± 0.1626	976
		10/16	6.4865 ± 0.0652	666

* Mean expressed as the natural log of amoeba number, used to calculate analysis of variance (Table 4B).

TABLE 4B. One-way analysis of variance by date and horizon, data transformed to ln (see Table 4 & 4A).

				TOTAL COUNT	
HORIZON	DATE	GROUPS	DF	MS	F
ORGANIC	6/16	among	2	2.40448451	
		within	21	0.195513657	12.2982944 **
	7/21	among	2	2.91406441	
		within	21	0.297946612	9.78049184 **
	8/25	among	2	1.06634045	
		within	21	0.375109809	2.8427421 NS
	9/9	among	2	0.606076002	
		within	21	0.24555556	2.4681827 NS
	10/16	among	2	0.678887606	
		within	21	0.35320282	1.92208999 NS
MINERAL	6/16	among	2	2.8685081	
		within	21	0.0929734593	30.8529781 **
	7/21	among	2	2.69171047	
		within	21	0.095415821	28.199471 **
	8/25	among	2	0.807935238	
		within	21	0.433789389	1.8625057 NS
	9/9	among	2	0.0832192898	
		within	21	0.163310119	0.50957828 NS
	10/16	among	2	0.113612771	
		within	21	0.0691672153	1.64258149 NS
CYST COUNT					
ORGANIC	6/16	among	2	0.517348528	
		within	21	0.307073911	1.68476875 NS
	7/21	among	2	0.005292892	
		within	21	0.208329655	0.02540633 NS
	8/25	among	2	0.539353371	
		within	21	0.511861597	1.05370939 NS
	9/9	among	2	0.131192923	
		within	21	0.289171537	0.45368546 NS
	10/16	among	2	0.023557663	
		within	21	0.304350263	0.07740313 NS
MINERAL	6/16	among	2	0.0665438175	
		within	21	0.0572151002	1.16304642 NS
	7/21	among	2	0.190997362	
		within	21	0.238764059	0.79994184 NS
	8/25	among	2	0.0729467869	
		within	21	0.225205535	0.32391205 NS
	9/9	among	2	0.0701990128	
		within	21	0.149363858	0.46998660 NS
	10/16	among	2	0.62591465	
		within	21	0.058220852	10.8138484 **

* = 5% significance level

** = 1% significance level

TABLE 5. SOIL MOISTURE (% w/w)¹:

HORIZON:	CONTROL SITE		ANTENNA SITE		GROUND SITE	
	ORG	MIN	ORG	MIN	ORG	MIN
DATE:						
6/16	21.0±5.4	10.4±1.9	29.9±10	7.4±1.2	25.0±6.6	11.3±2.2
7/21	29.7±7.5	18.1±2.2	34.8±7.7	14.5±2.6	30.6±7.1	18.6±3.4
8/25	30.8±7.8	15.0±1.7	34.3±11.1	11.3±3.6	31.8±7.4	14.6±1.9
9/9	34.3±6.6	14.0±2.8	30.5±6.4	10.4±1.8	32.7±7.4	14.4±2.7
10/16	35.3±10	15.6±0.5	28.9±4.3	11.8±0.7	41.6±6.5	16±0.8

ONE-WAY ANOVA:

		ORGANIC		MINERAL	
Date		D.F.	M.S.	D.F.	M.S.
6/16	Between	2	156.88	2	33.66
	Within	21	58.72	21	3.29
	F=	2.6717	NS	10.2446	**
7/21	Between	2	3.49	2	40.37
	Within	21	50.57	21	7.705
	F=	0.069	NS	5.2399	*
8/25	Between	2	26.55	2	32.49
	Within	21	79.77	21	6.57
	F=	0.332	NS	4.943	*
9/9	Between	2	24.468	2	38.51
	Within	21	46.316	21	6.087
	F=	0.528	NS	6.327	**
10/16	Between	2	324.555	2	38.89
	Within	21	53.1596	21	0.509
	F=	6.10529	**	76.3982	**

¹ Sample size, 8

* = 5% significance level

** = 1% significance level

Table 6. Regression calculations for growth of Acanthamoeba polyphaga and uptake of bacteria, log transformed.

Experiment [*]	Slope ^{**}	95% Confidence Limits ^{***}
Amoeba growth 1	0.04817	L1 = 0.04 L2 = 0.06
Amoeba growth 2	0.08399	L1 = 0.06 L2 = 0.11
Bacteria uptake 1	-5.32927	L1 = -0.011 L2 = 4.197
Bacteria uptake 2	-9.42261	L1 = -0.018 L2 = -1.512

* Amoeba & bacteria 1: counts at 0,3,6,21 & 24 hr; 20 counts each.

Amoeba & bacteria 2: counts at 0,4,18 & 24 hr; 30 counts each.

** Mean Generation Time = 7 hr.

*** For the slope of the curve.

Table 7. Isoenzyme analysis, 1986 & 1987 seasons
 NEI'S 'D' (GENETIC DISTANCE) IS ABOVE THE DIAGONAL
 'I' (GENETIC IDENTITY) IS BELOW THE DIAGONAL
 'J(X)' (AVERAGE HOMOZYGOSITY) IS ON THE DIAGONAL

FROM SITE A (ANTENNA) IN 1986 SEASON.

	1	2	3	4	5	6	7	8	9	10
1 (.804)		.365	.393	.449	.387	.687	.737	.809	.705	.829
2 .694	(.765)		.243	.225	.362	.471	.568	.553	.587	.662
3 .675	.784	(.824)		.335	.18	.605	.488	.487	.624	.736
4 .638	.799	.715	(.794)		.33	.656	.798	.594	.675	.769
5 .679	.696	.835	.719	(.843)		.639	.423	.47	.592	.699
6 .503	.624	.546	.519	.528	(.775)		.344	.476	.331	.308
7 .478	.567	.614	.45	.655	.709	(.775)		.253	.519	.396
8 .445	.575	.615	.552	.625	.621	.777	(.804)		.487	.491
9 .494	.556	.536	.509	.553	.718	.595	.615	(.824)		.25
10 .436	.516	.479	.463	.497	.735	.673	.612	.778	(.814)	

FROM SITE G(GROUND) IN 1986 SEASON.

	1	2	3	4	5	6	7	8	9	10
1 (.784)		.575	.482	.454	.415	.699	.668	.743	1.034	.748
2 .563	(.873)		.526	.478	.517	.715	.673	.365	.559	.638
3 .617	.591	(.804)		.071	.311	.424	.511	.458	.754	.664
4 .635	.62	.932	(.775)		.259	.415	.451	.411	.639	.6
5 .66	.596	.733	.772	(.775)		.572	.534	.605	.978	.692
6 .497	.489	.654	.66	.564	(.755)		.146	.57	.528	.513
7 .513	.51	.6	.637	.586	.865	(.765)		.587	.514	.395
8 .476	.694	.633	.663	.546	.566	.556	(.824)		.315	.459
9 .356	.572	.47	.528	.376	.59	.598	.729	(.843)		.248
10 .473	.528	.515	.549	.501	.599	.674	.632	.78	(.853)	

FROM SITE C (CONTROL) IN 1986 SEASON.

	1	2	3	4	5	6	7	8	9	10
1 (.863)		.429	.357	.468	.798	.575	.664	.637	.517	.6
2 .651	(.794)		.507	.465	.482	.421	.408	.687	.675	.693
3 .7	.603	(.833)		.647	.849	.776	.806	.785	.589	.562
4 .626	.628	.524	(.814)		.611	.443	.497	.525	.587	.845
5 .45	.617	.428	.543	(.794)		.392	.372	.543	.565	.612
6 .562	.656	.46	.642	.676	(.745)		.568	.716	.766	.964
7 .515	.665	.446	.608	.689	.567	(.814)		.27	.229	.426
8 .529	.503	.456	.592	.581	.489	.763	(.843)		.142	.391
9 .597	.509	.555	.556	.569	.465	.796	.868	(.863)		.29
10 .549	.5	.57	.43	.542	.381	.653	.676	.749	(.853)	

Table 7. Continued

NEI'S "D" (GENETIC DISTANCE) IS ABOVE THE DIAGONAL
 "I" (GENETIC IDENTITY) IS BELOW THE DIAGONAL
 "J(X)" (AVERAGE HOMOZYGOSITY) IS ON THE DIAGONAL

FROM SITE A (ANTENNA) IN 1987 SEASON.

1	2	3	4	5	6	7	8	9	10
1 (.912)	.419	.416	.78	.643	.892	.59	.881	.404	.372
2 .658	(.892)	.389	.721	.845	.801	.579	.733	.51	.518
3 .659	.678	(.873)	.54	.6	.532	.578	.633	.233	.236
4 .458	.486	.583	(.843)	.15	.457	.531	.331	.693	.631
5 .526	.43	.549	.86	(.843)	.535	.454	.468	.541	.509
6 .41	.449	.588	.633	.586	(.814)	.513	.459	.554	.705
7 .554	.56	.561	.588	.635	.599	(.824)	.512	.433	.546
8 .415	.481	.531	.718	.626	.632	.599	(.863)	.765	.834
9 .668	.6	.792	.5	.582	.575	.649	.465	(.824)	.259
10 .689	.596	.79	.532	.601	.494	.579	.434	.772	(.853)

FROM SITE G (GROUND) IN 1987 SEASON.

1	2	3	4	5	6	7	8	9	10
1 (.873)	.623	.536	.676	.293	.394	.566	.458	.307	.305
2 .536	(.882)	.769	.984	.411	.602	.582	.411	.36	.405
3 .585	.463	(.853)	.441	.643	.575	.262	.659	.594	.699
4 .509	.374	.643	(.824)	.647	.809	.579	.597	.812	.854
5 .746	.663	.526	.523	(.863)	.301	.472	.426	.255	.472
6 .674	.547	.563	.445	.74	(.873)	.468	.495	.38	.543
7 .568	.559	.77	.56	.624	.626	(.804)	.553	.493	.625
8 .632	.663	.517	.55	.653	.609	.575	(.833)	.482	.312
9 .736	.697	.552	.444	.775	.684	.611	.618	(.833)	.402
10 .737	.667	.497	.426	.624	.581	.535	.732	.669	(.882)

FROM SITE C (CONTROL) IN 1987 SEASON.

1	2	3	4	5	6	7	8	9	10
1 (.863)	.89	.59	.632	.474	.526	.267	.851	.267	.311
2 .41	(.833)	.562	.753	.867	.489	.83	.635	.989	.971
3 .554	.57	(.853)	.331	.598	.373	.554	.299	.616	.62
4 .531	.471	.718	(.853)	.676	.315	.595	.582	.534	.642
5 .622	.42	.55	.509	(.824)	.564	.405	.513	.517	.405
6 .591	.613	.688	.73	.569	(.814)	.52	.601	.636	.533
7 .766	.436	.575	.552	.667	.594	(.853)	.681	.239	.282
8 .427	.53	.741	.559	.599	.548	.506	(.814)	.766	.564
9 .766	.372	.54	.586	.597	.53	.787	.465	(.853)	.144
10 .733	.379	.538	.526	.667	.587	.755	.569	.866	(.824)

STATISTICS FOR GENETIC DISTANCE (1986) ^a

Site	Mean genetic distance + SD
A	0.5108 ± 0.175
B	0.5589 ± 0.178
C	0.5314 ± 0.186

One-way ANOVA : (for site A, B and C)

	d.f.	M.S.
Among	2	0.0262
Within	132	0.0322

F = 0.8134 (NS)

^aAbbreviations : SD, standard deviation; ANOVA, analysis of variance; d.f., degree of freedom; M.S., mean square; F, F-test; NS, not significant at the 0.05 level.

STATISTICS FOR GENETIC DISTANCE (1987) ^a

Site	Mean genetic distance + SD
A	0.5494 ± 0.175
B	0.5558 ± 0.198
C	0.5229 ± 0.167

One-way ANOVA : (for site A, B and C)

	d.f.	M.S.
Among	2	0.01369
Within	132	0.03257

F = 0.4204 (NS)

^aAbbreviations : SD, standard deviation; ANOVA, analysis of variance; d.f., degrees of freedom; M.S., mean square; F, F-test; NS, not significant at the 0.05 level.

Two-way ANOVA ^a

Source of variation	df	M.S.	F
Site	2	MS1=0.02488	MS1/MS4=0.7672 NS
Year	1	MS2=5.4676E-03	MS2/MS4=0.1686 NS
Site x Year	2	MS3=0.01496	MS3/MS4=0.4613 NS
Experiment error	264	MS4=0.03243	

^aAbbreviations: ANOVA, analysis of variance; df, degree of freedom; M.S., mean square; F, F-test; NS, not significant at the 0.05 level.

Figure 1. Control site total counts and cyst counts.

CONTROL SITE (1987)

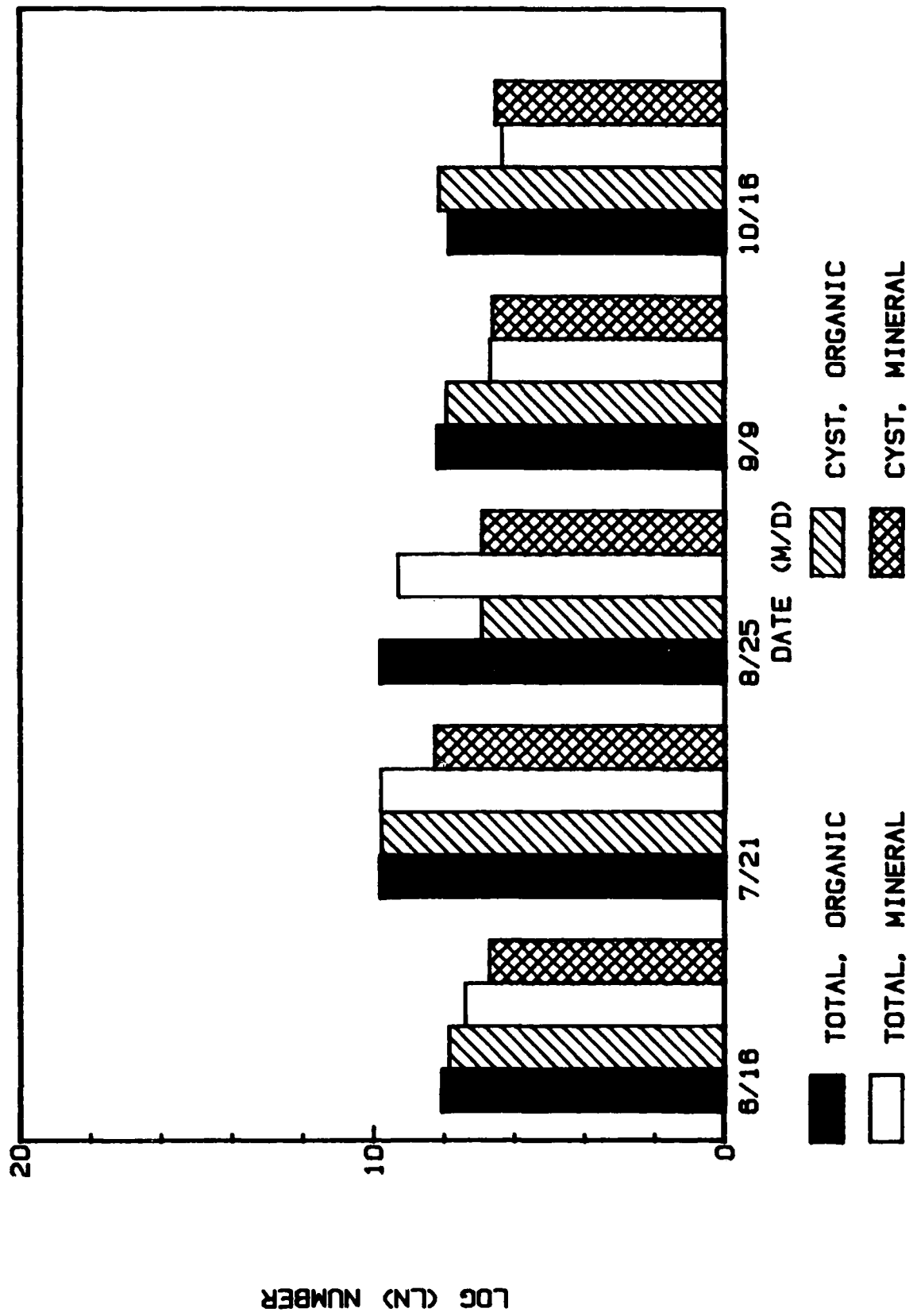


Figure 2. Antenna site total counts and cyst counts.

ANTENNA SITE (1987)

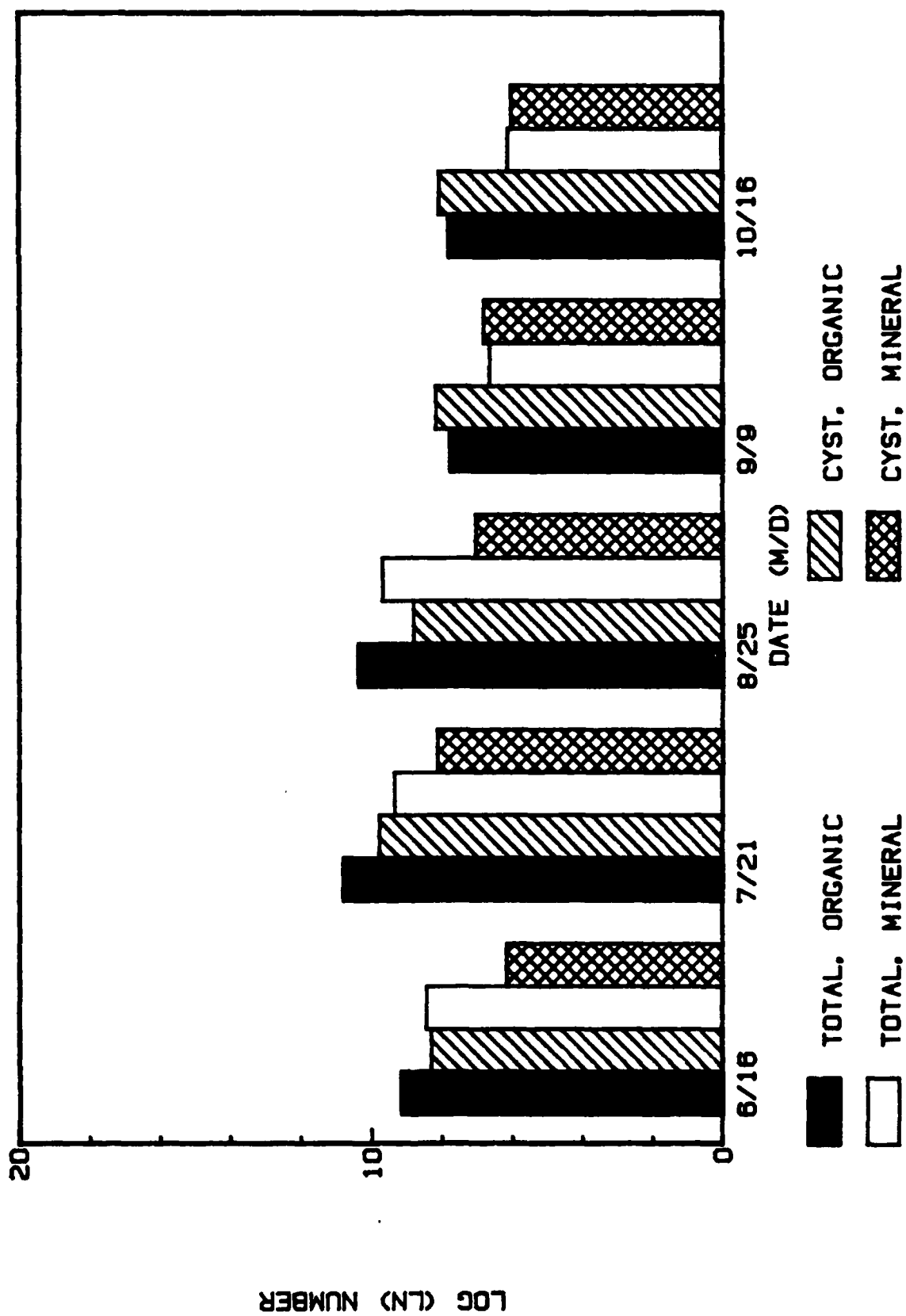


Figure 3. Ground site total counts and cyst counts.

GROUND SITE (1987)

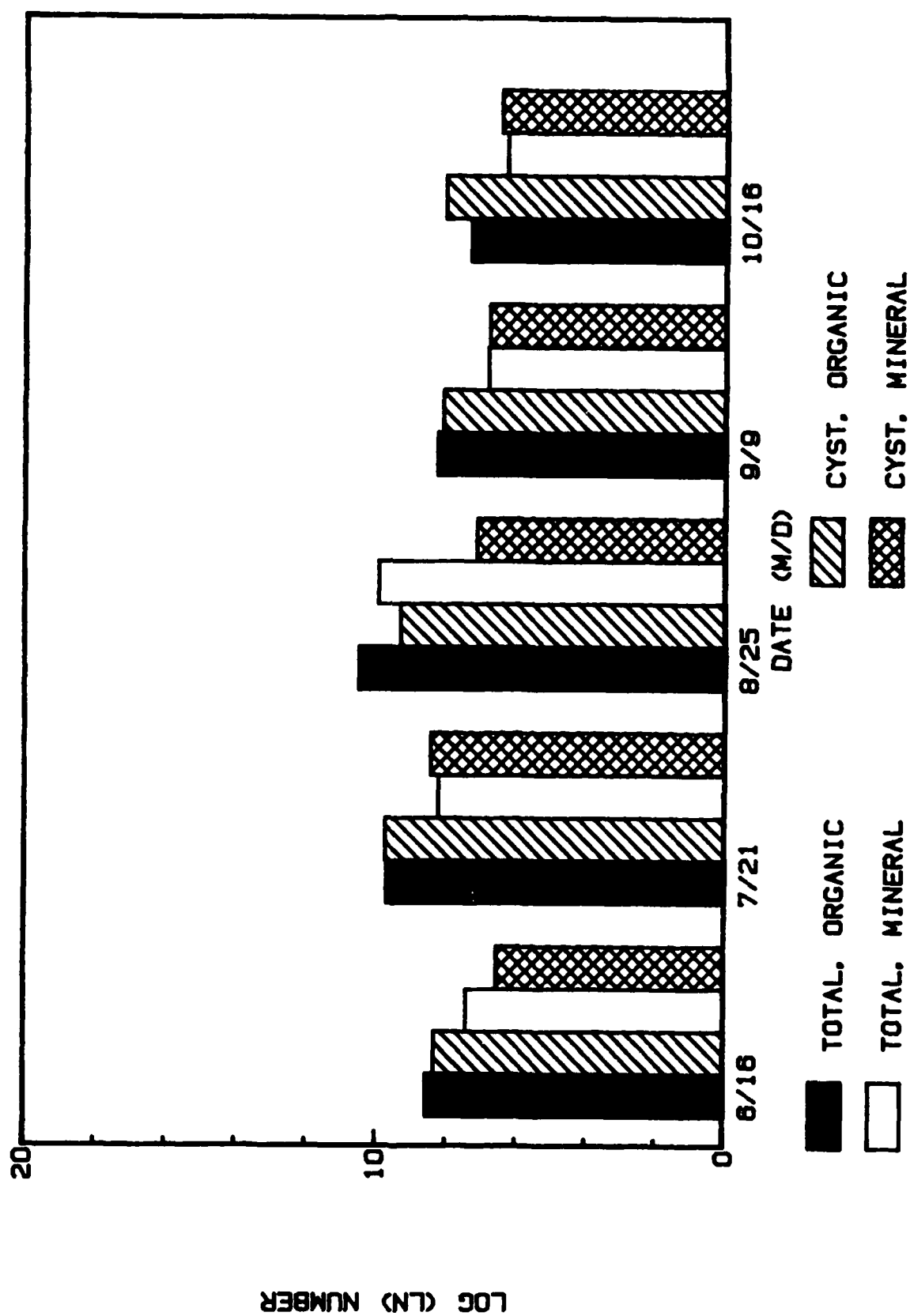


Figure 4. Average total number of ameobae at the 3 sites, ORGANIC HORIZON.

ORGANIC HORIZON (1987)

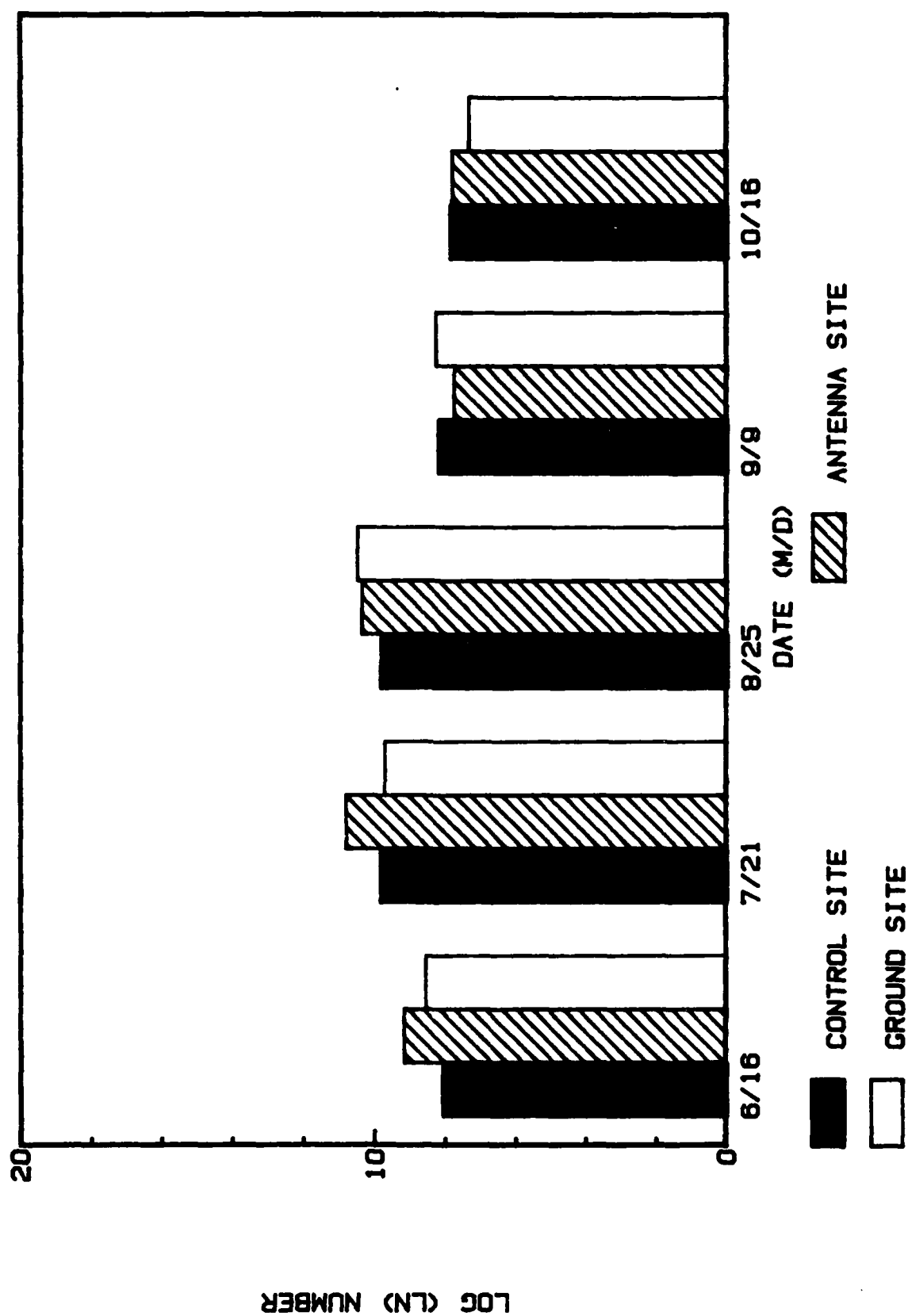


Figure 5. Average number of amoebae at the 3 sites, MINERAL HORIZON.

MINERAL HORIZON (1987)

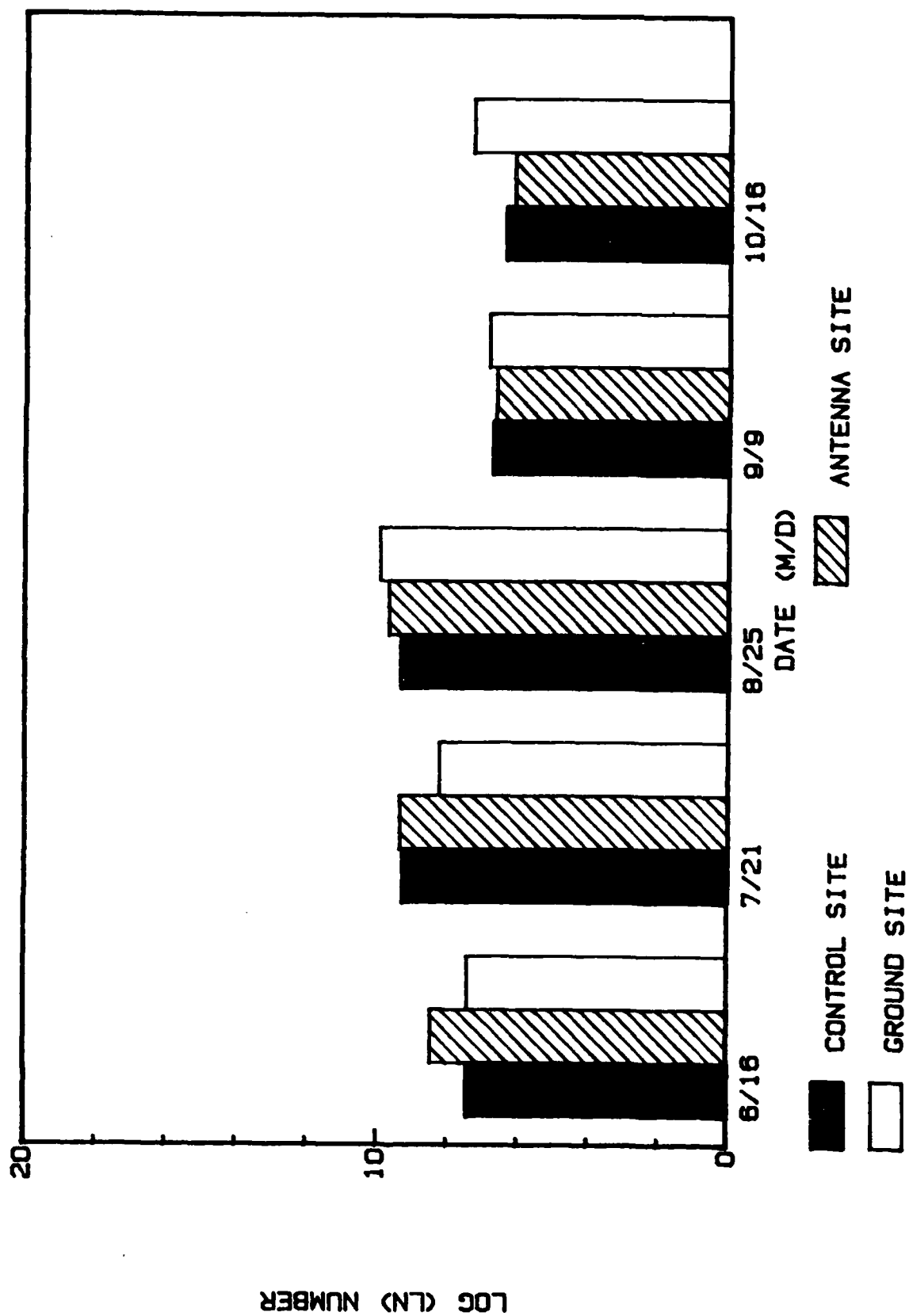


Figure 6. Calculated number of vegetative amoebae from Table 4 and 4A determined by subtracting means.

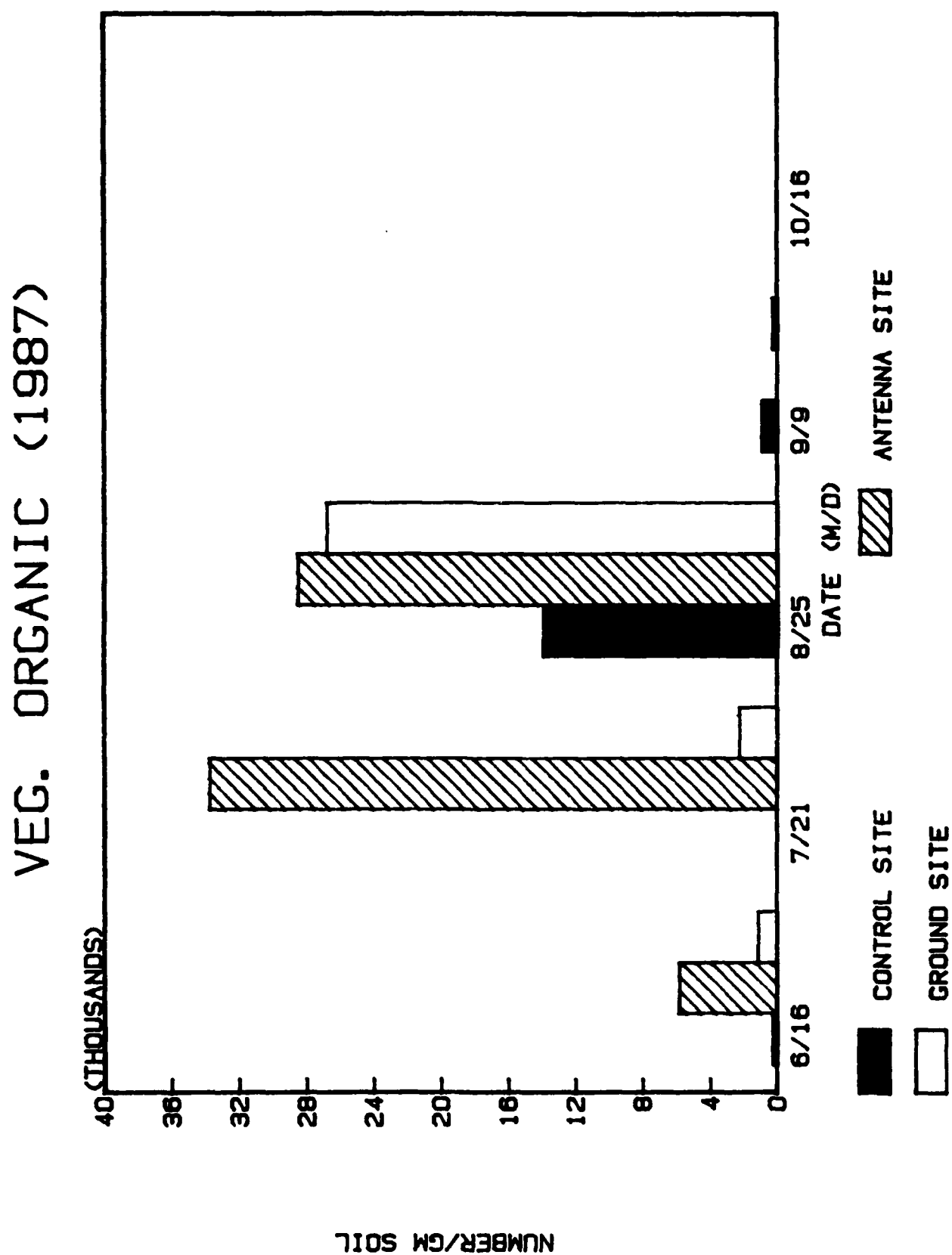


Figure 7. Calculated number of vegetative amoebae from Table 4 and 4A determined by subtracting means.

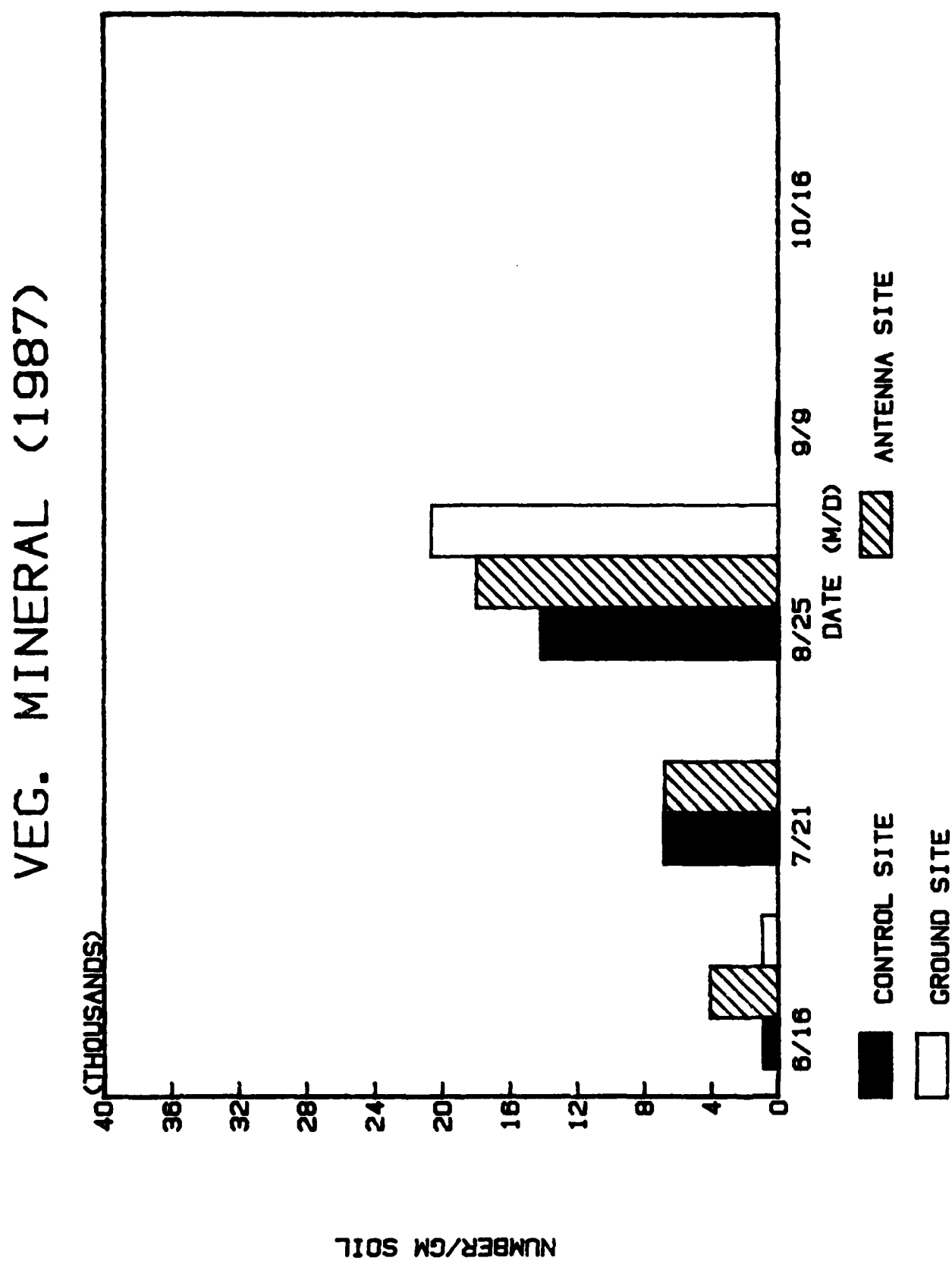


Figure 8. Percent vegetative amoebae, ORGANIC HORIZON.

VEG. ORGANIC (1987)

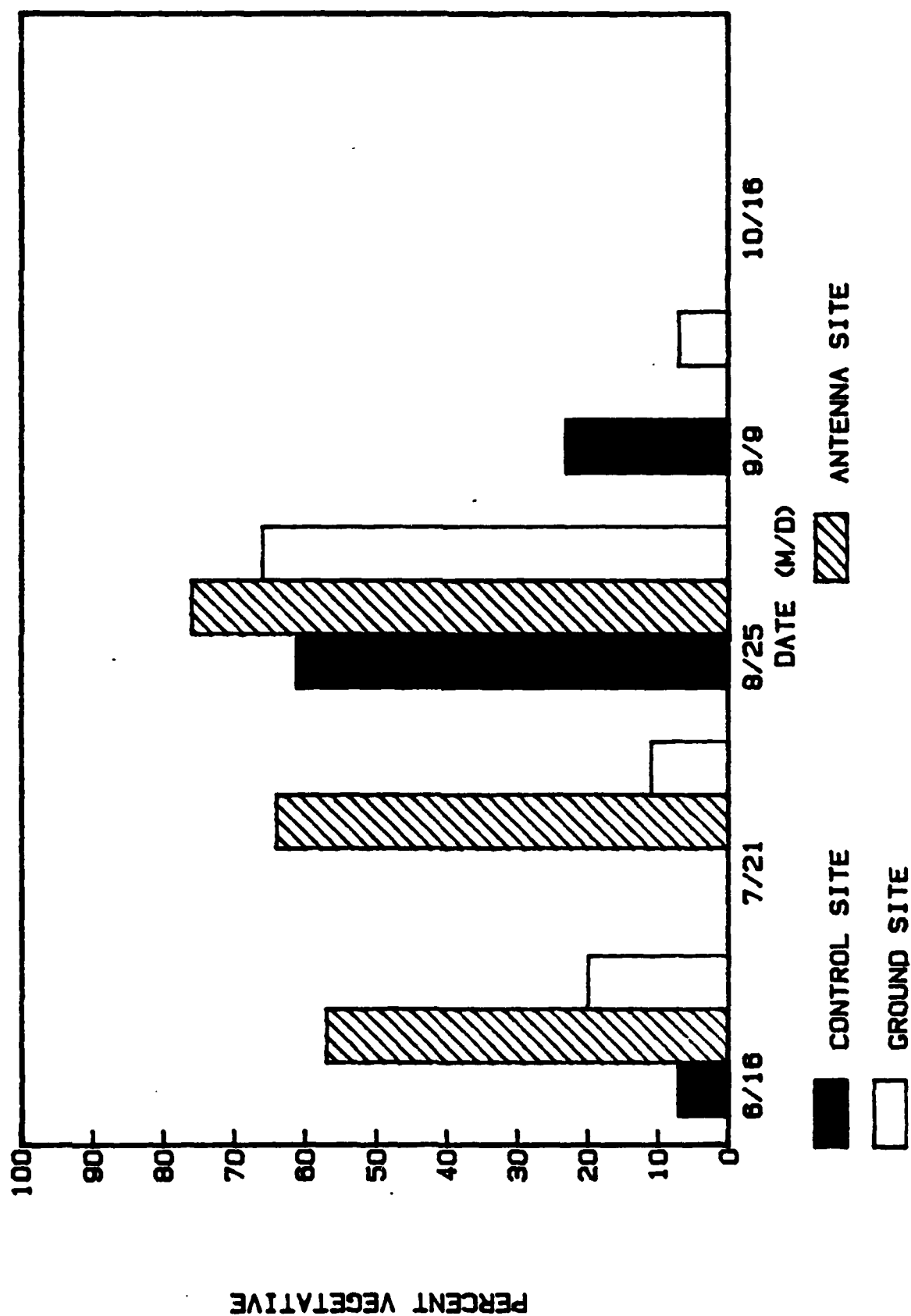


Figure 9. Percent vegetative amoebae, MINERAL HORIZON.

VEG. MINERAL (1987)

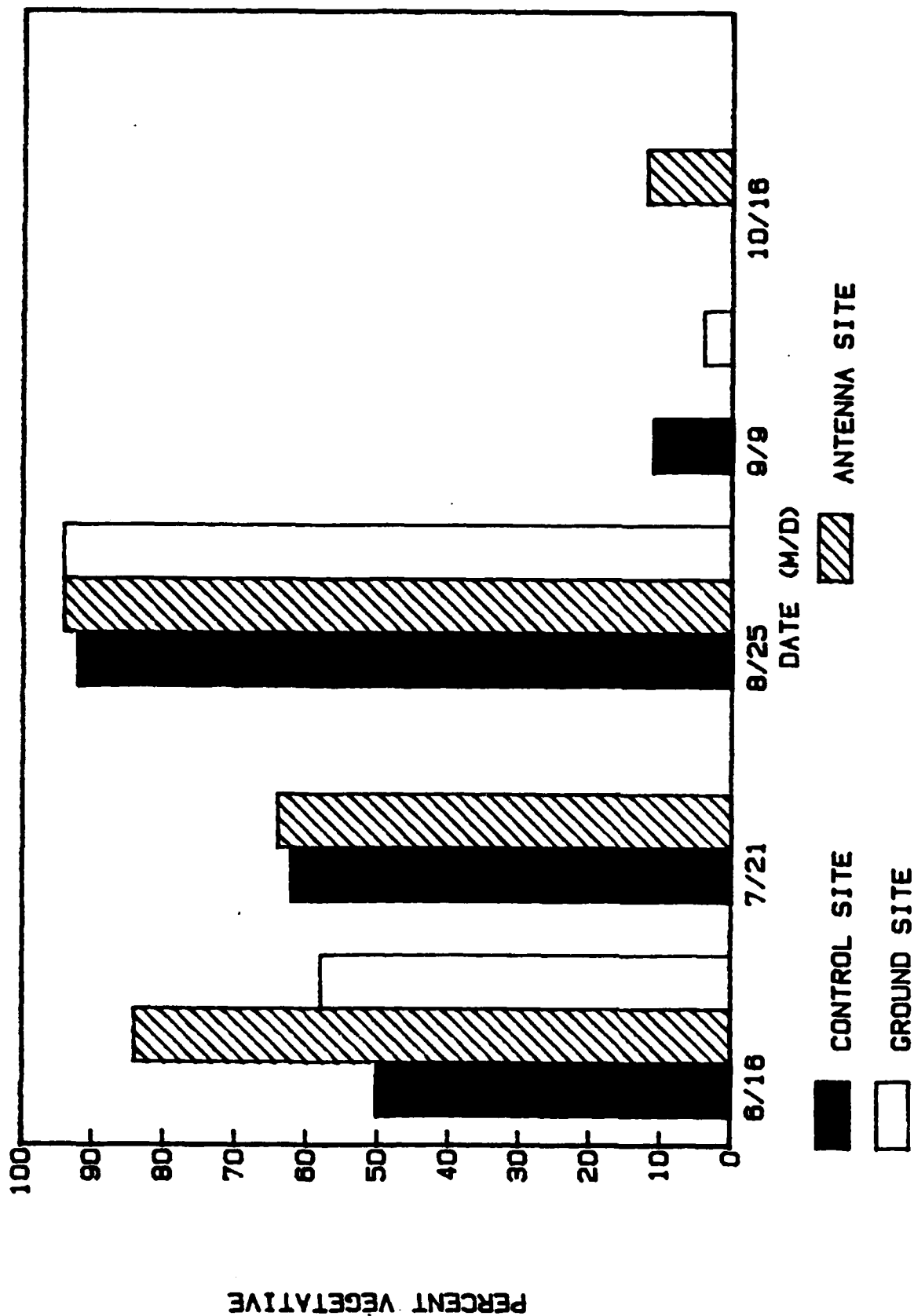


Figure 10. Total number of amoebae per gram of soil, ORGANIC HORIZON.

ORGANIC HORIZON (1987)

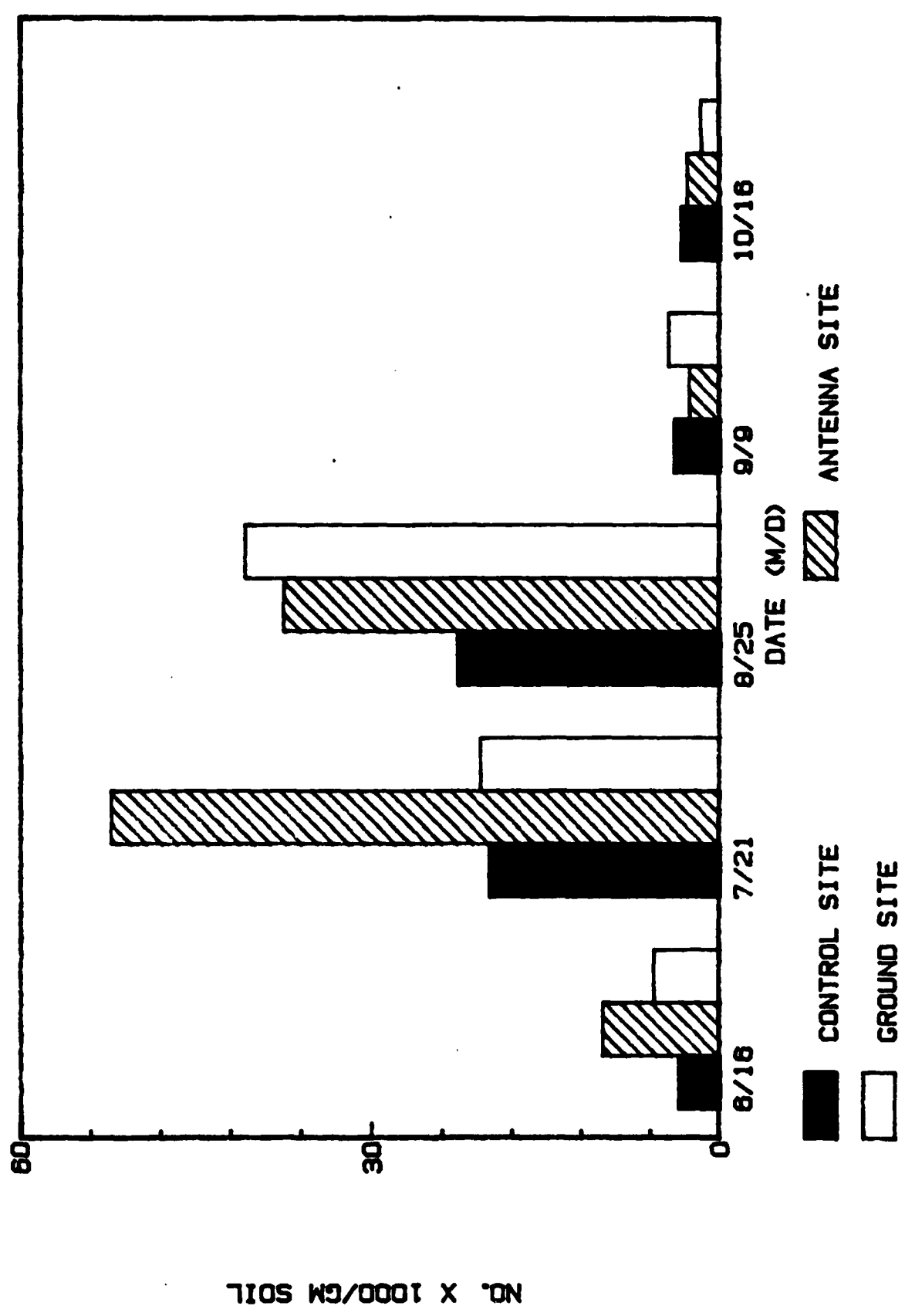


Figure 11. Total number of amoebae per gram of soil, MINERAL HORIZON.

MINERAL HORIZON (1987)

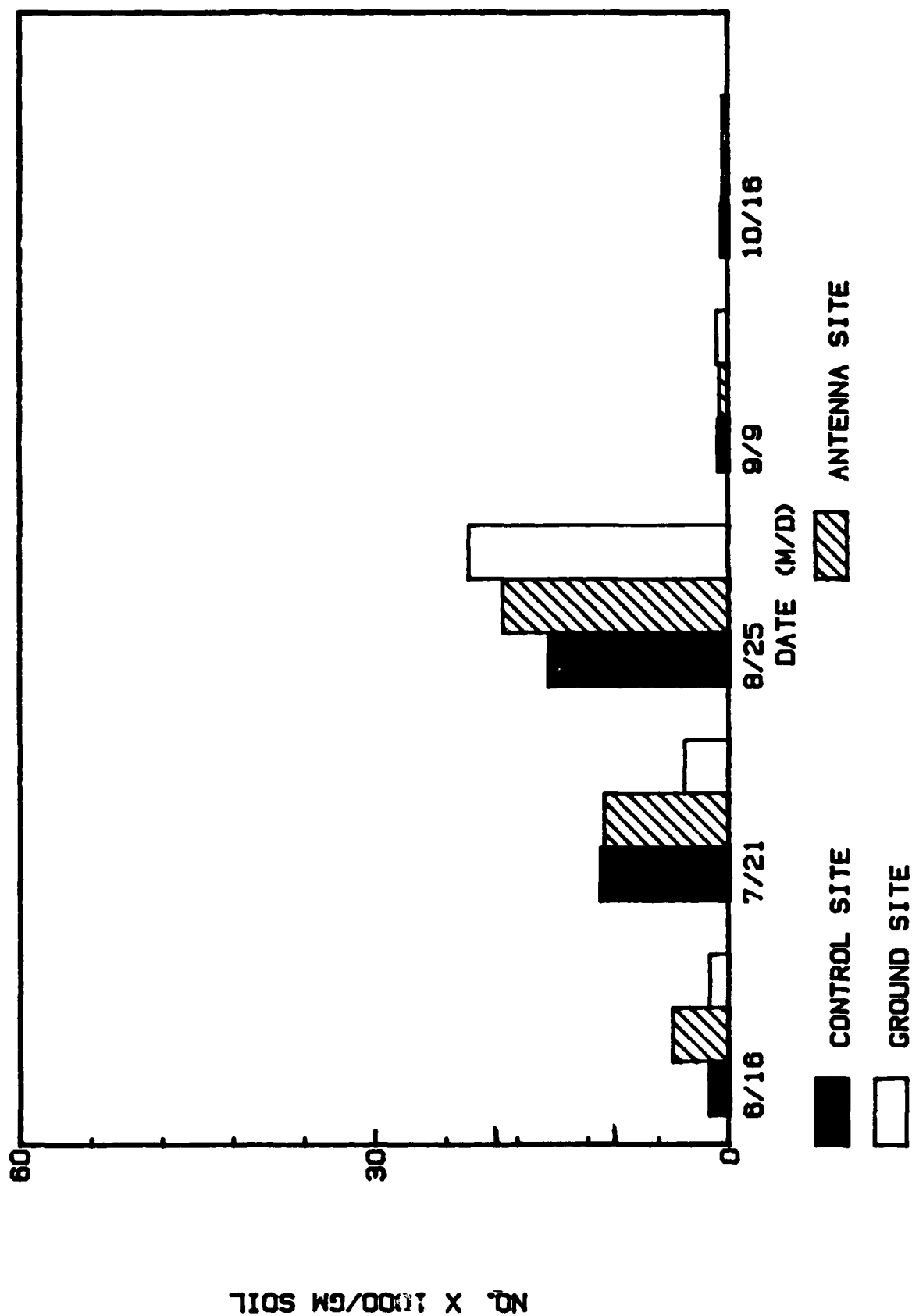


Figure 12. Moisture content of samples taken for counting, ORGANIC HORIZON.

ORG. HORIZON MOISTURE

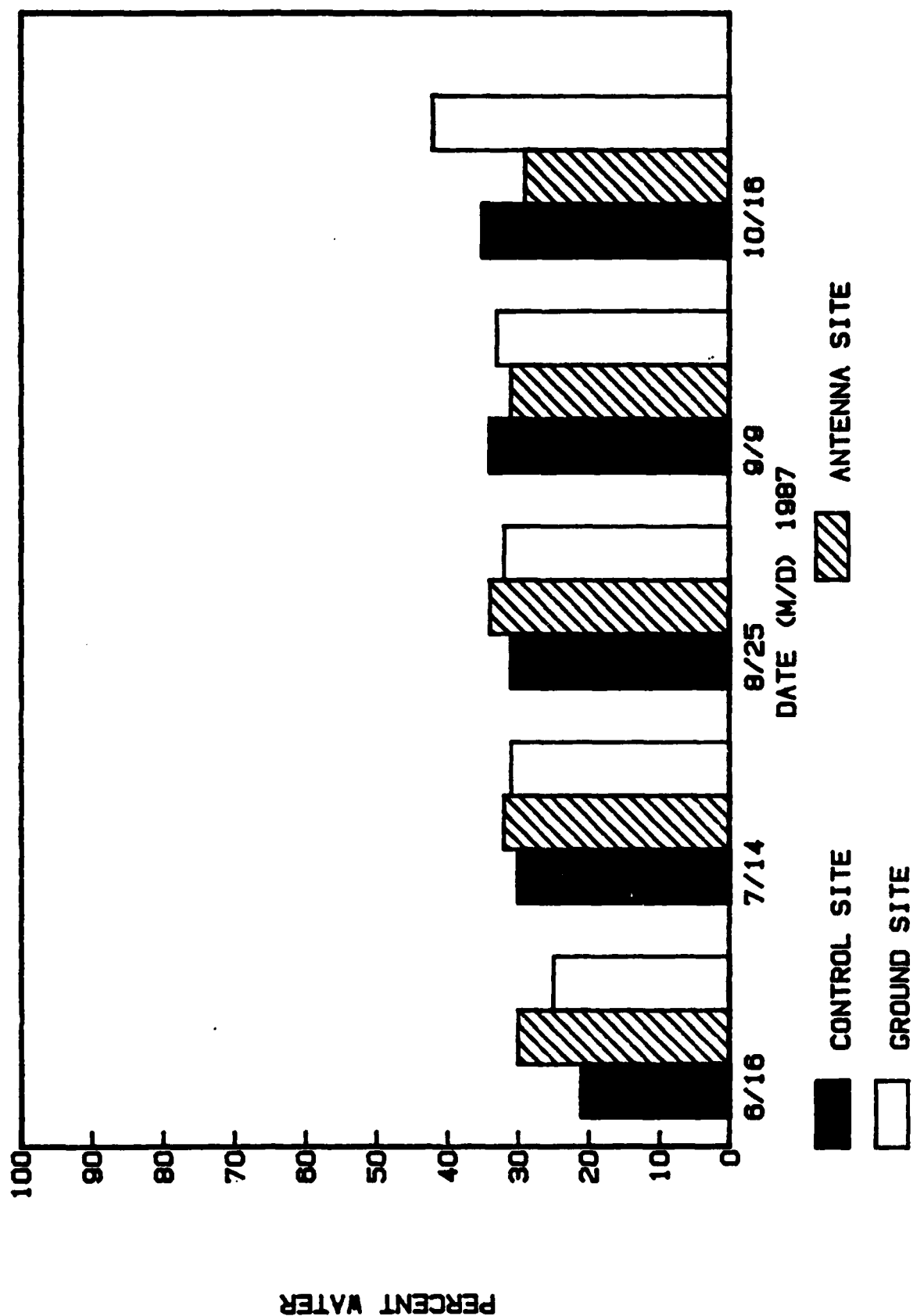


Figure 13. Moisture content of samples taken for counting, MINERAL HORIZON.

MIN. HORIZON MOISTURE

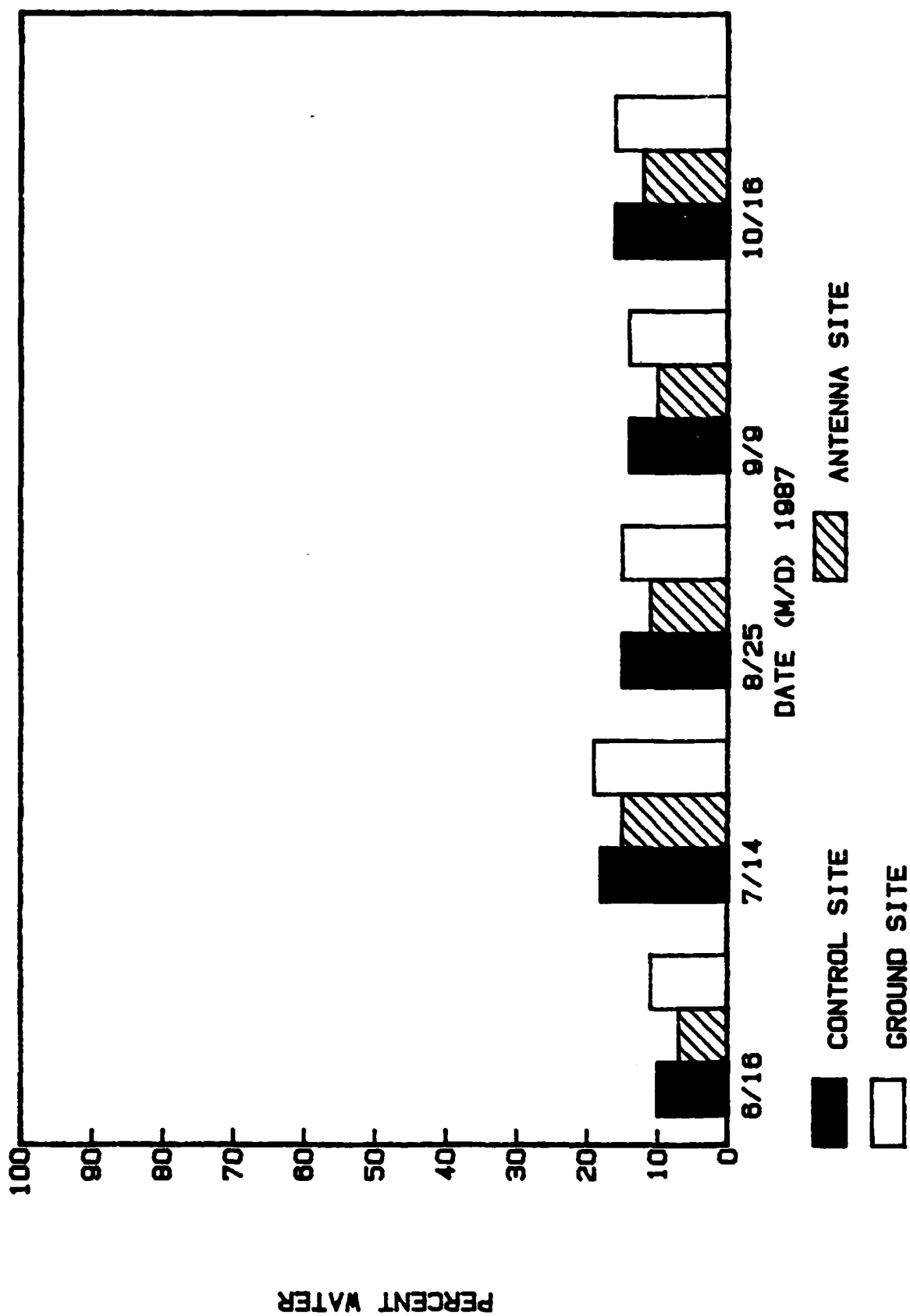


Figure 14. Annual rainfall by month for 3 seasons vs. average rainfall for 1951 to 1980 (i.e. normal). Data excerpted from the Climatological Data for Michigan, published by NOAA.

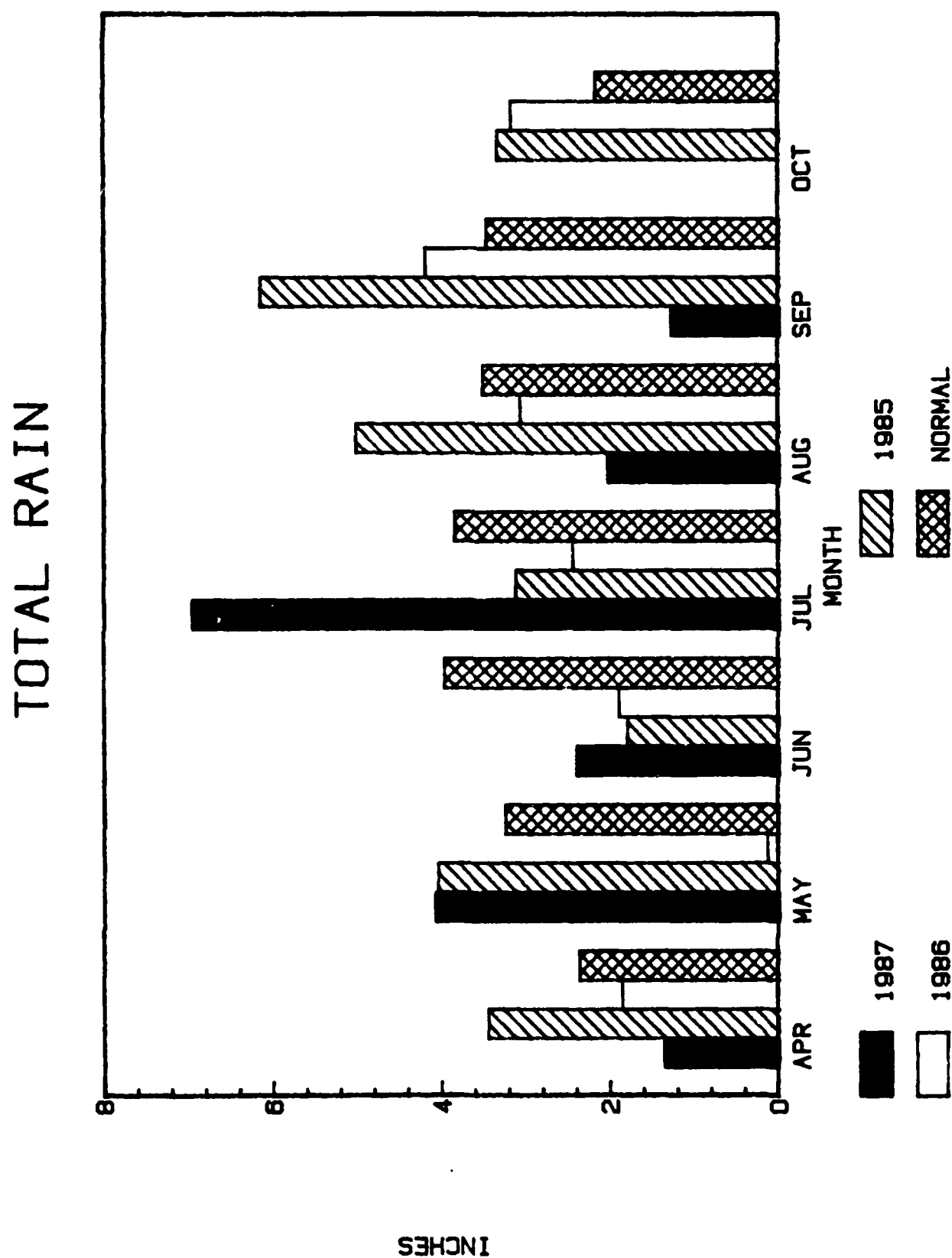


Figure 15. Pooled temperature records, mineral horizon, all sites, mean daily temperatures with S.D. error bars. Points plotted every third day.

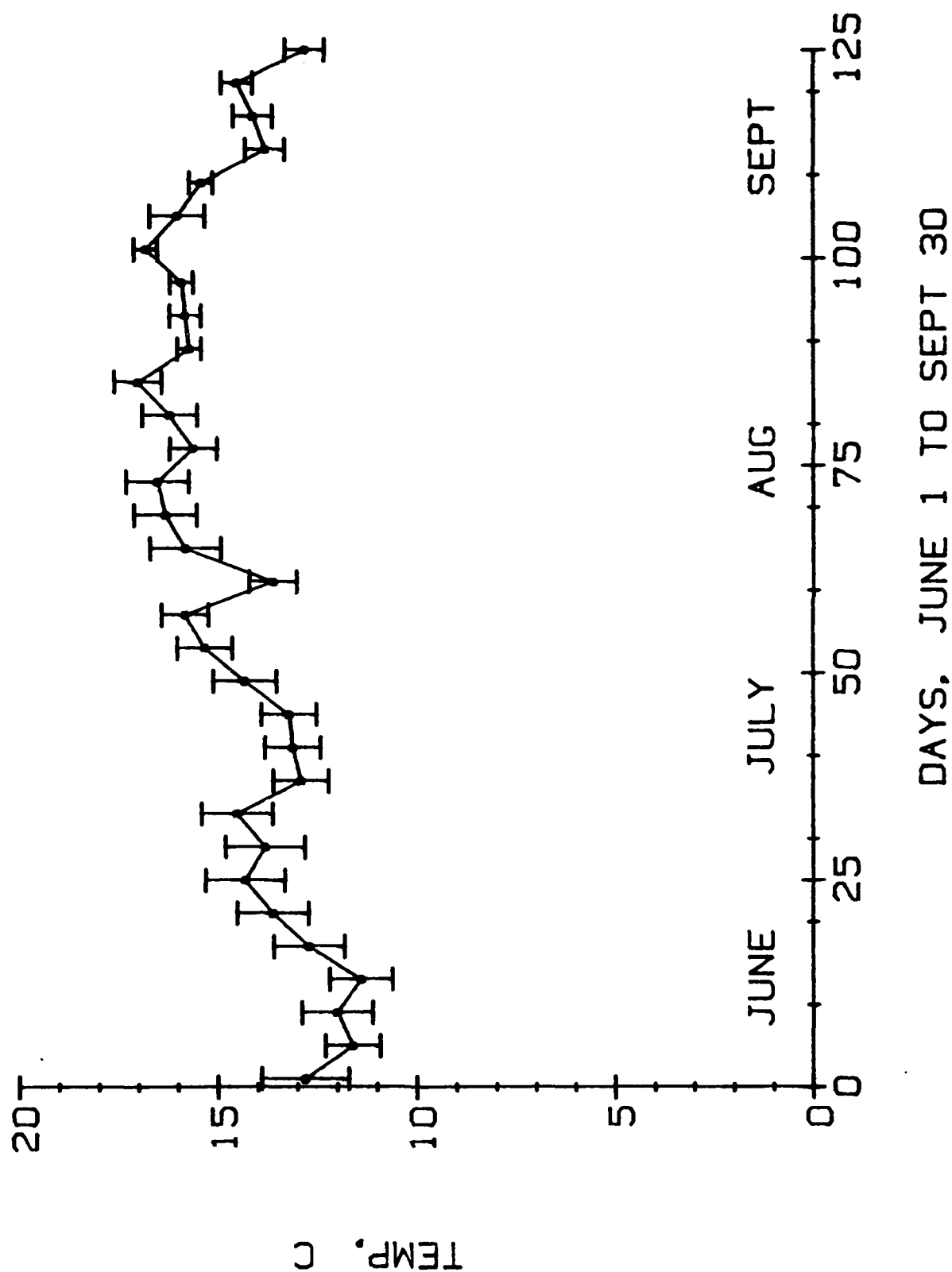


Figure 16. Growth rate of amoebae at two different concentrations.
(Mean generation time 6-7 hours). (Y-axis: Log numbers).

ACANTHAMEOBA POLYPHAGA

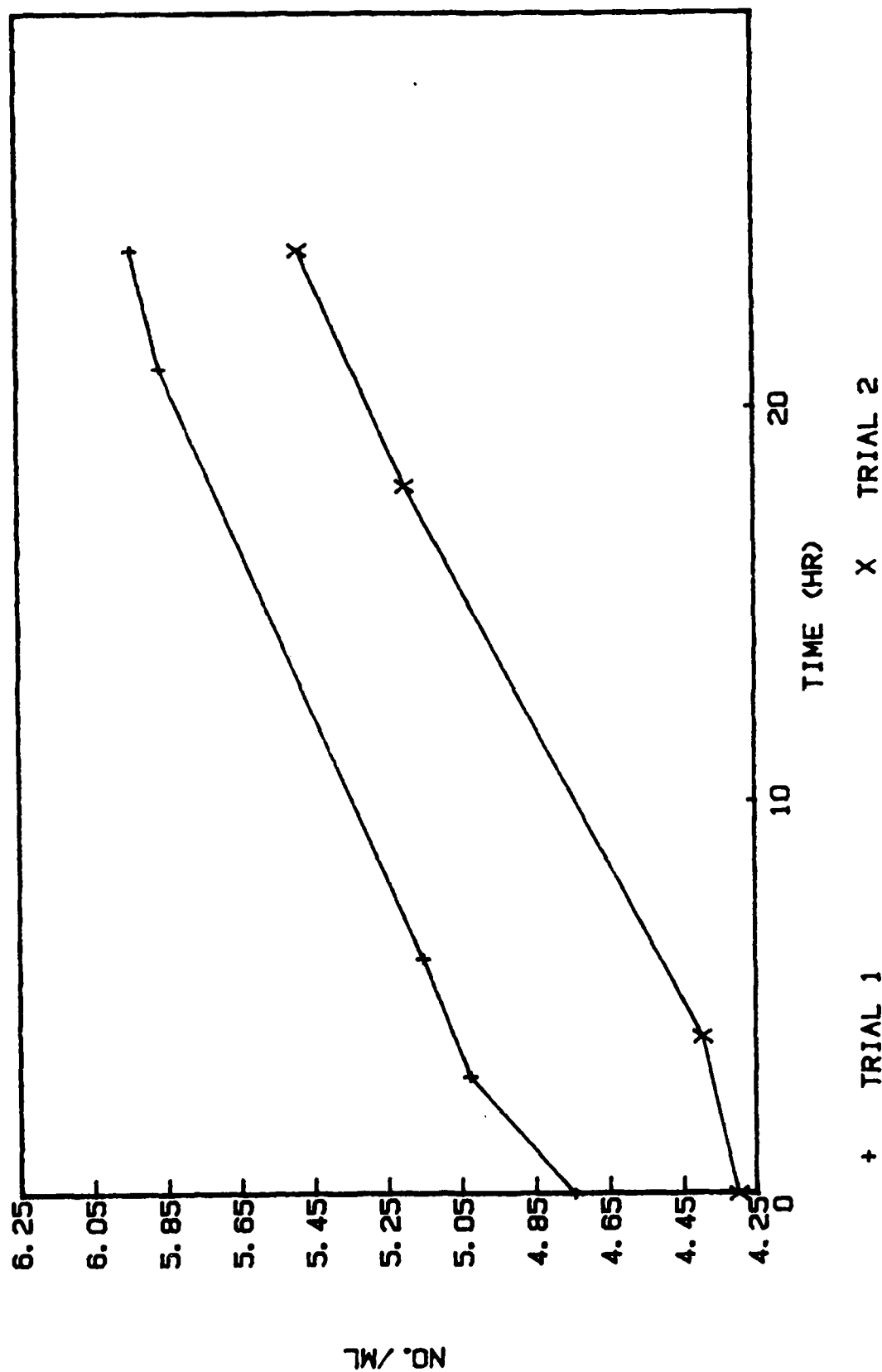


Figure 17. Bacterial uptake of amoebae at two densities of E. coli.
(Y-axis: Log numbers).

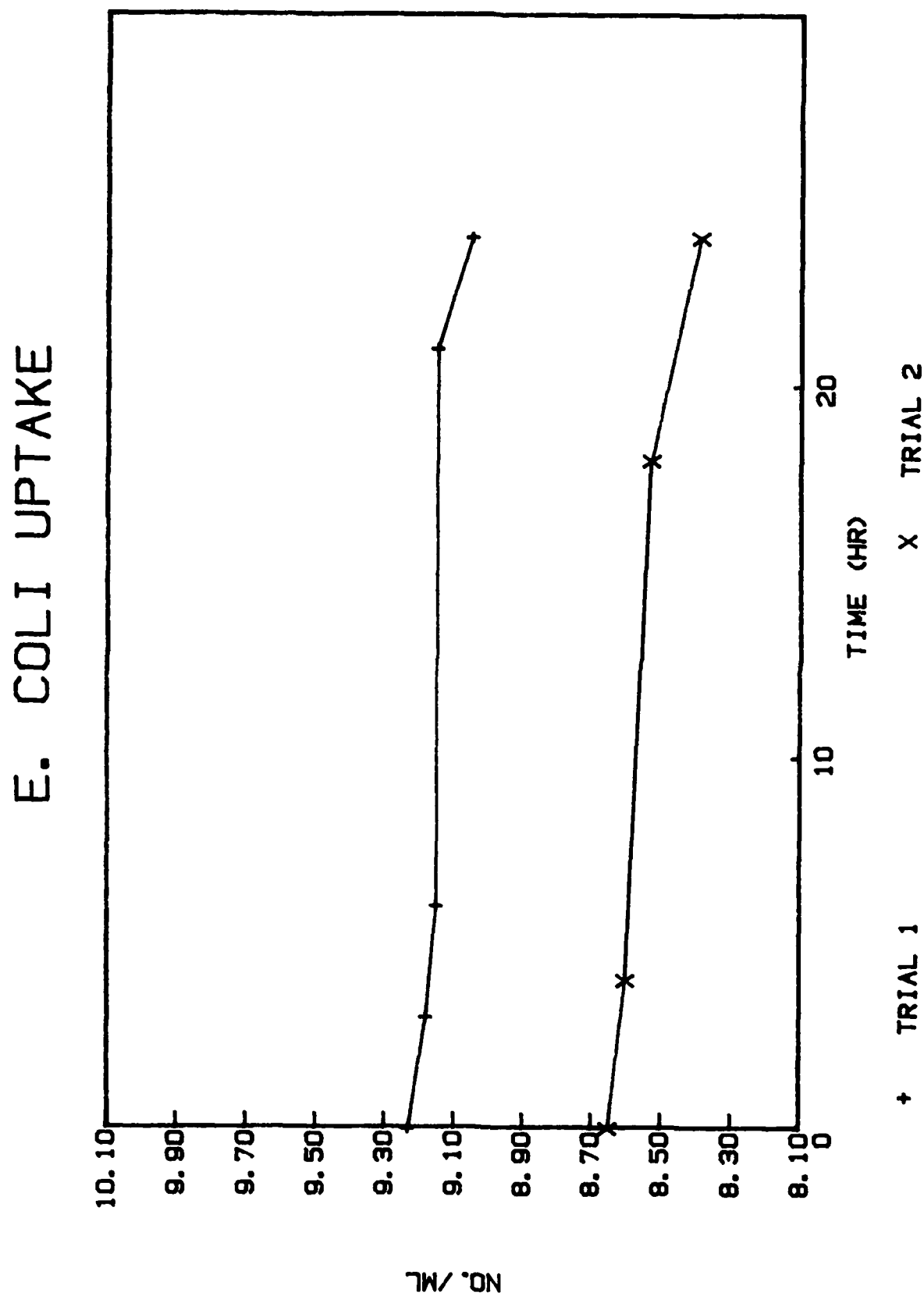


Figure 18. Number of bacteria consumed per amoeba as the density of bacteria decreases in the culture, per 2 hour interval.

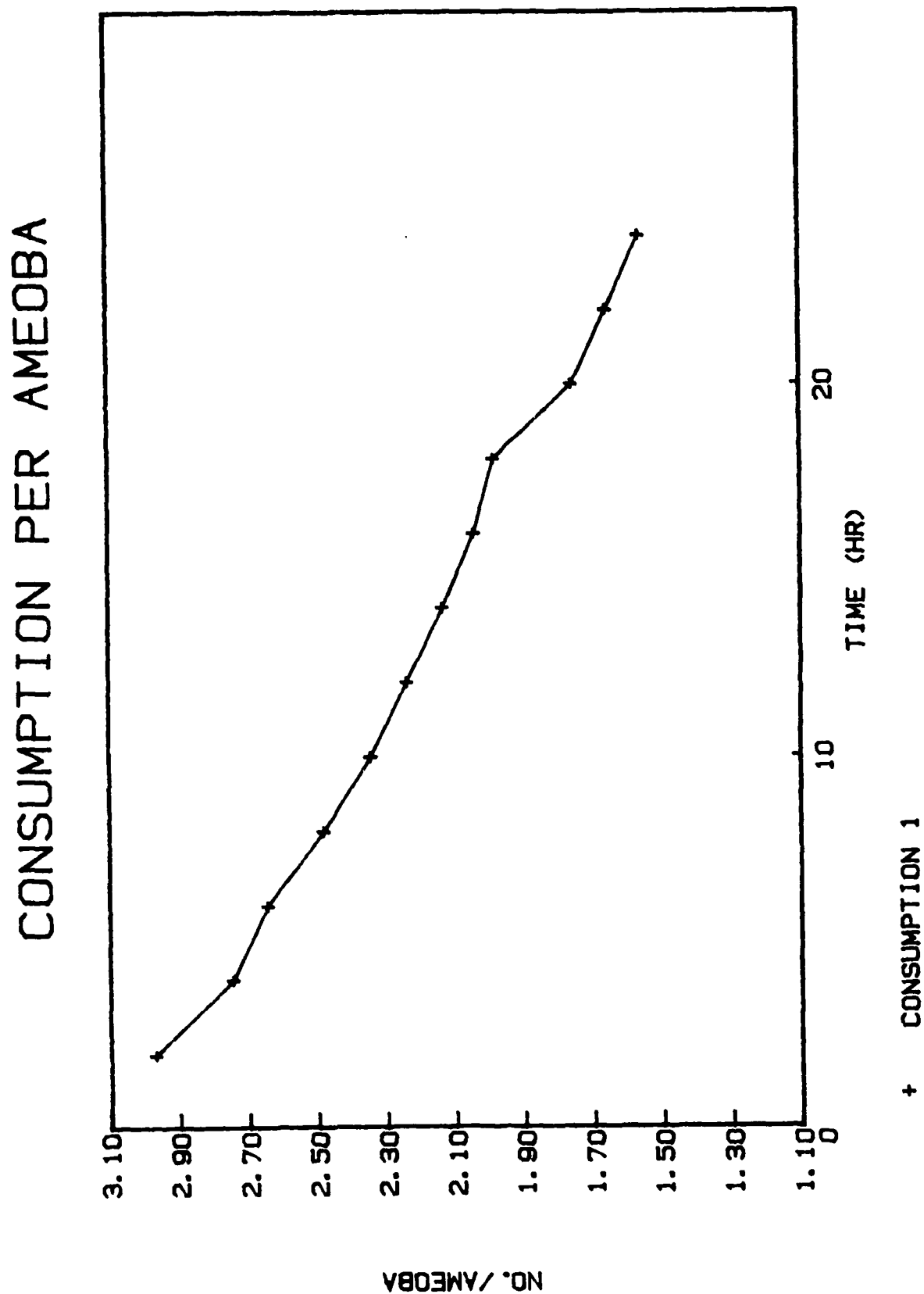


Figure 19. Consumption of bacteria per amoeba vs. varying densities of bacteria.

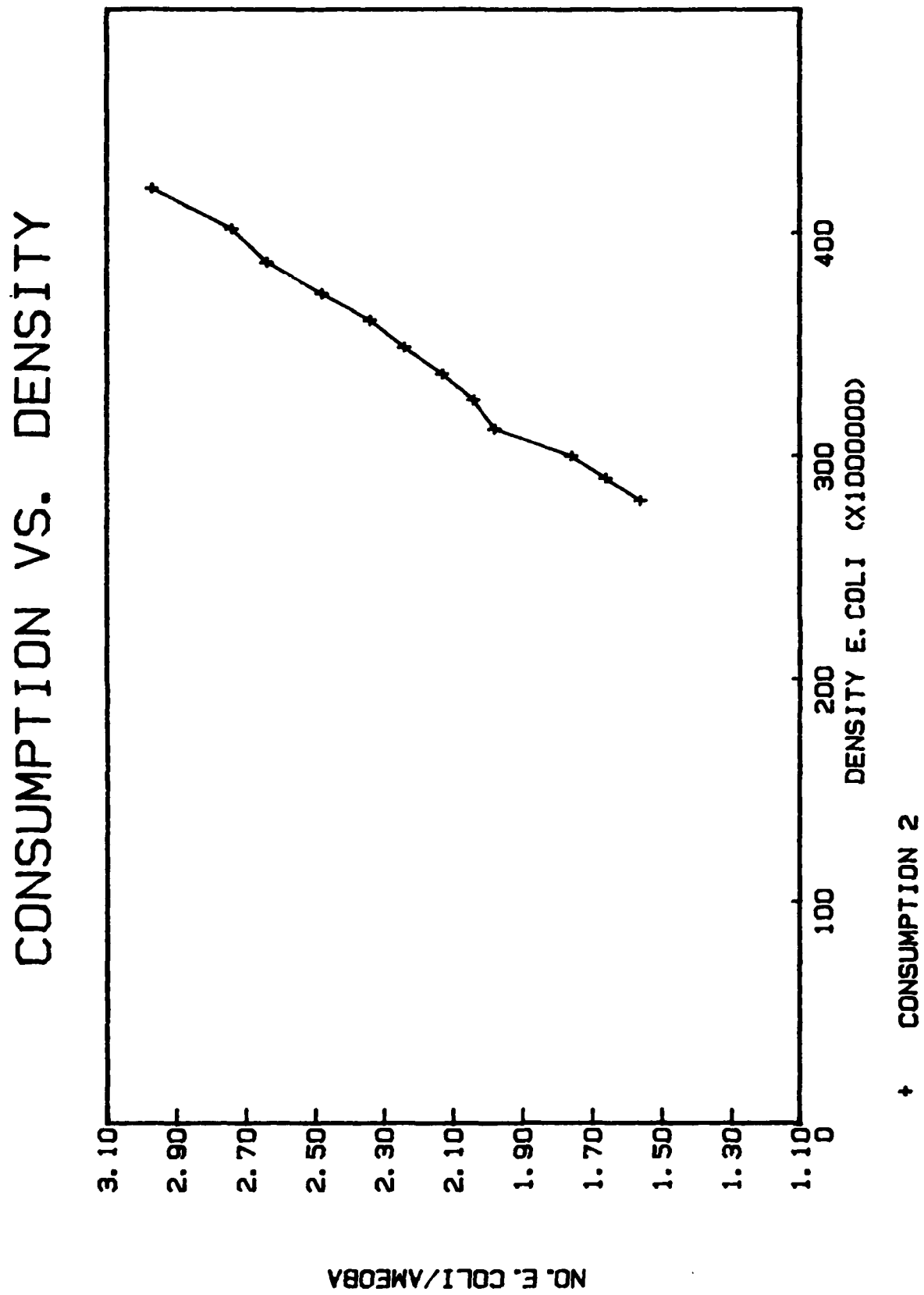


Figure-20. Summary of 1984 amoeba counts, given both as log counts and absolute numbers, % vegetative amoebae and % soil water.

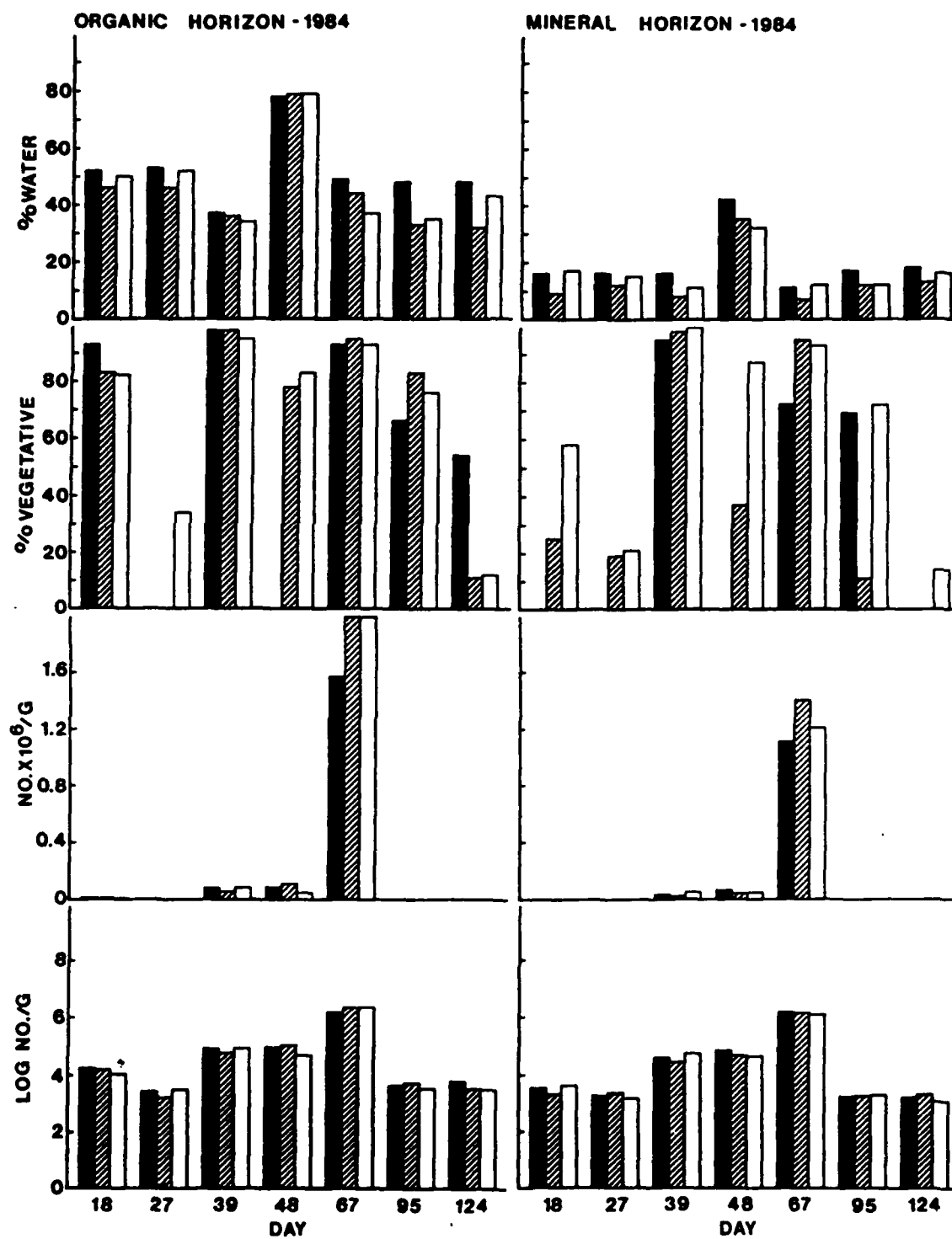


Figure 21. Summary of 1985 amoeba counts, given both as log counts and absolute numbers, % vegetative amoebae and % soil water.

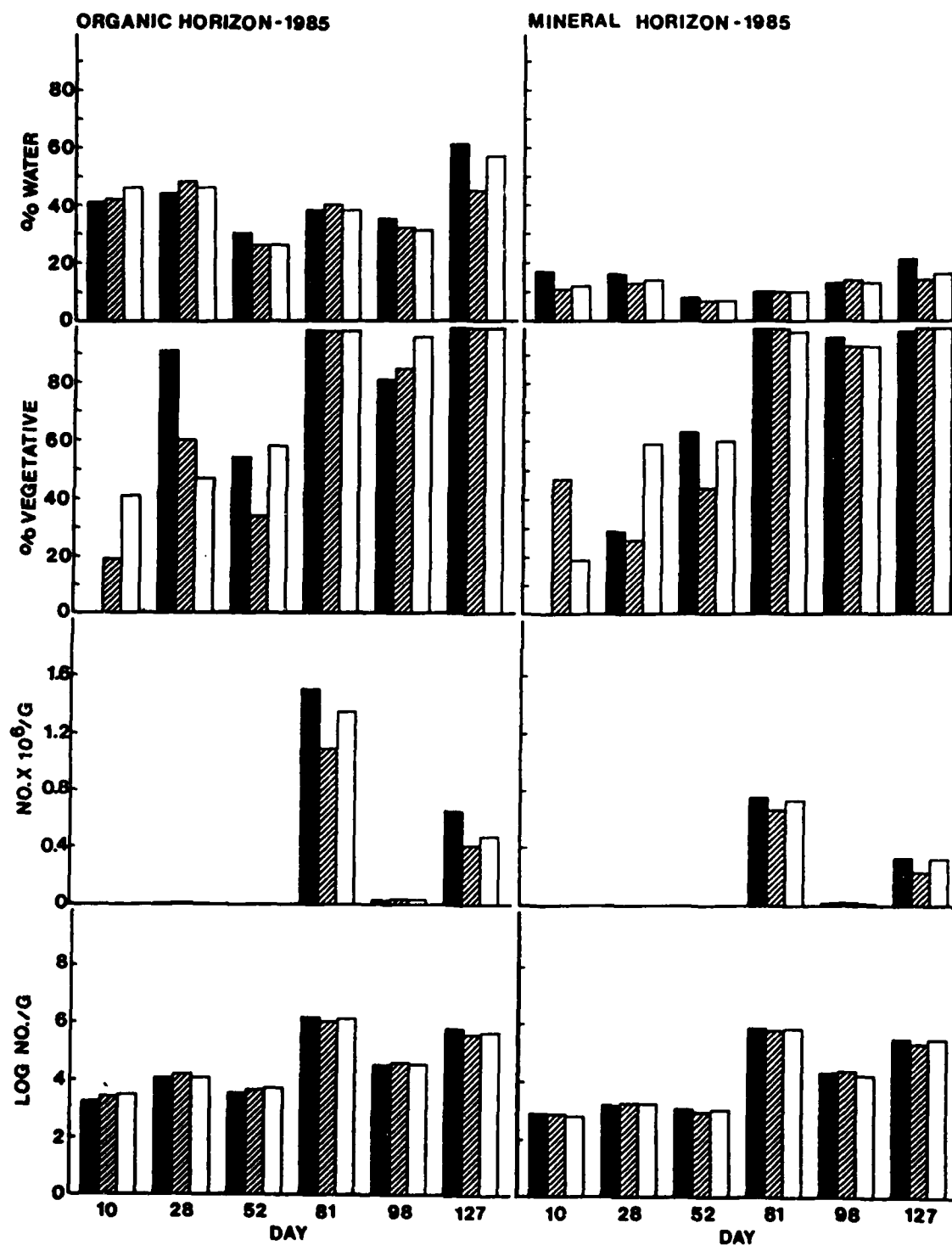


Figure 22. Summary of 1986 amoeba counts, given both as log counts and absolute numbers, % vegetative amoebae and % soil water. Note that the absolute number scale differs from previous-years.

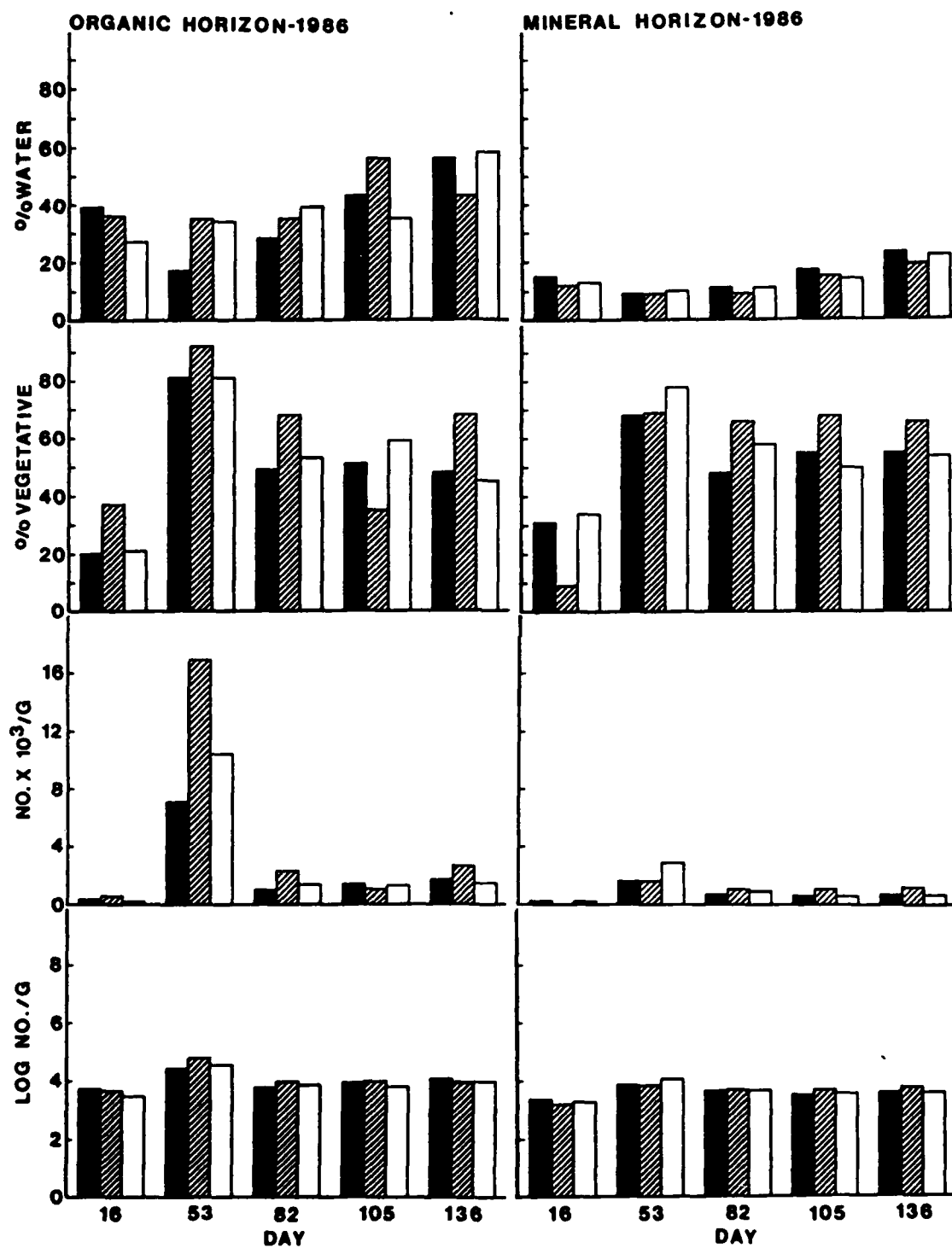
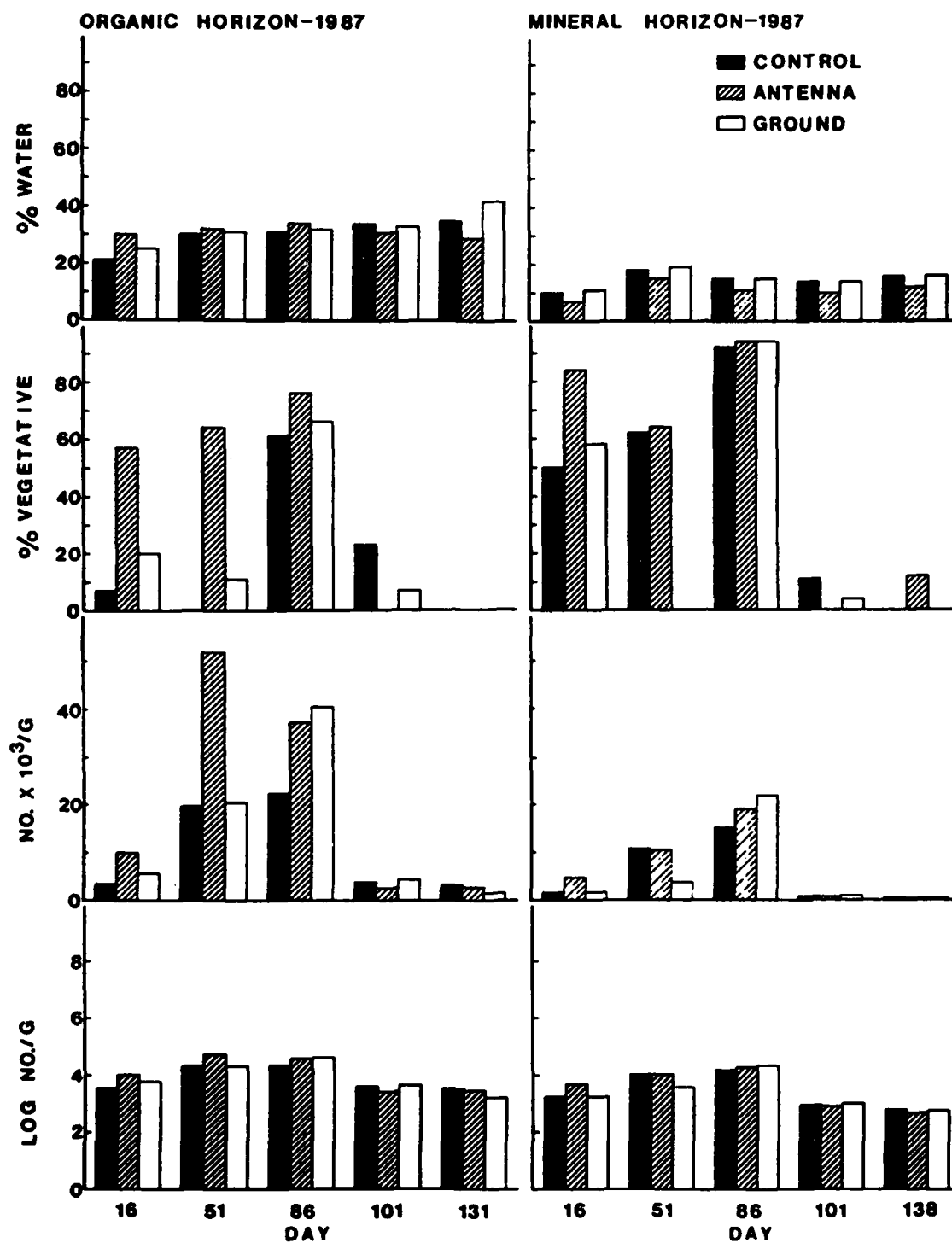


Figure 23. Summary of 1987 amoeba counts, given both as log counts and absolute numbers, % vegetative amoebae and % soil water.



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Arthropoda and Earthworms

(Tasks 5.3. and 5.4.)

Annual report

1987

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East Lansing, Michigan 48824

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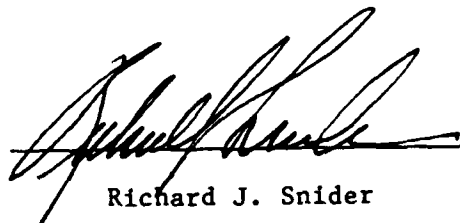
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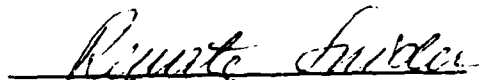
Soil and Litter Arthropoda and Earthworm Studies

Tasks 5.3. and 5.4.

1987



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TABLE OF CONTENTS

Abstract	1
Summary	3
I. REVIEW.....	7
II. ENVIRONMENTAL MONITORING	9
1. Precipitation	9
2. Litter and soil moisture	11
3. Temperature	14
III. SOIL AND LITTER ARTHROPODA	19
1. Extraction efficiency for Collembola	19
2. Collembolan community structure	20
3. Collembolan abundance	23
3.1. Family-level comparisons	23
3.2. Densities of common species	23
3.3. Analysis of seasonal densities	25
3.4. Population structure of <u>Isotoma notabilis</u>	32
4. Acari	33
IV. SURFACE-ACTIVE ARTHROPODA	49
1. Collembola	49
1.1. Numbers and diversity	49
1.2. Seasonal activity	51
2. Carabidae	56
2.1. Dominance and diversity	56
2.2. Seasonal activity	58
2.3. Diel activity	58
2.4. Fecundity	63
V. LUMBRICIDAE	65
1. Community structure	65
2. Horizontal distribution	67
2.1. Distribution over quadrats	67
2.2. Horizontal distribution of epigeics	69
3. Vertical distribution	70
4. Density and biomass	77
5. Reproduction	80

6. Cocoons	85
6.1. Cocoon weights	85
6.2. Cocoon production	88
6.3. Cocoon density, development and emergence	89
7. Weight class distribution	95
VI. LITTER INPUTS AND DECOMPOSITION	99
1. Litter inputs	99
2. Litter standing crops	99
3. Turnover rates of forest floor litter	101
4. Litter mass loss	102
5. ANOVA of mass loss in large-mesh bags	105
6. Analysis of single leaf mass loss	106
7. Proposed work	111
VII. GENERAL CONCLUSIONS	115
REFERENCES CITED	119
APPENDIX A: Publications and manuscripts	123

ABSTRACT

With minor deviations, sampling schedules and protocols of earlier years were again adhered to in 1987 (early May to late October). Data analysis was intensified since three full years (1984-86) of pre-ELF documentation were at hand for most arthropod and lumbricid taxa monitored. Results allowed some conclusions with respect to invertebrate populations of Test and Control: seasonal abundances usually differed significantly between years and sites; careful choice and formatting of environmental covariates will be needed if analysis of seasonal population fluctuations is to reach any useful level of sensitivity for site comparison. However, trends in population density as well as community diversity should be monitored in the future in order to quantify the potential year-to-year variation in these parameters.

At a higher level of resolution, population attributes such as seasonal occurrence and frequency of developmental stages, reproductive activity, fecundity and vertical distribution allowed relatively sensitive site comparison. In addition, surface-active arthropods showed responses to air temperatures which were species-specific and quantifiable, within-year differences between sites being non-significant as measured by regression analysis.

Leaf litter inputs and forest floor standing crops did not differ between sites, over the 1984-87 period. Turnover rates of natural litter, as well as mass loss of confined litter samples, were shown to be useful process-oriented parameters for long-term monitoring of Test and Control.

SUMMARY

Arthropod and earthworm populations inhabiting leaf litter and soil were sampled, as in previous years, from early May to late October 1987. Rainfall, temperature (air and soil), and substrate moisture were monitored concurrently.

Data stemming from 1984 to 1986 (the pre-ELF period) were subjected to various statistical analyses. Identification of ecological parameters which did not differ between Test and Control sites, or which were relatively constant between years, was one of the major goals. By way of summary, three major categories of data bases will be distinguished here:

1. Overall numbers of animals as they fluctuated with season or from year to year: in general, these data were not sensitive to detection of potential changes. Populations of earthworms, and particularly of arthropods, varied from year to year in both sites. In some species, rainfall patterns and resulting soil or litter moisture had distinct effects on population trends. Ongoing analyses are taking these environmental variables into account in the form of covariates, in order to increase the explanatory power of population analyses.

2. Population attributes other than total numbers or diversity were found to be more useful for project goals. They included:

- a. Behavioral traits: vertical distribution of litter- as well as soil-dwelling earthworms did not differ significantly between sites, being governed mainly by moisture conditions. Fluctuations in activity of arthropods (springtails and carabid beetles) in response to air temperature were essentially the same in both sites for a given species.

- b. Size structure of populations: in the earthworm Dendrobaena octaedra, for instance, frequencies of weight classes vary seasonally as

individuals hatch and grow; based on several thousand individual worm weights, weight frequency distributions over three years did not differ significantly between sites. In soil- and litter-dwelling mites, seasonal occurrence of egg-bearing females, larvae and subsequent immature stages also did not differ between sites, indicating close synchronicity of life cycle phenomena in Test and Control.

c. Reproduction: for carabid beetles, which are most active (and thus captured in traps) during their reproductive period, we have begun to quantify fecundity. Females were dissected and the developmental stage of their ovaries as well as the number of mature eggs in them were recorded. Preliminary data indicate that the average number of eggs carried per individual is species-specific and relatively constant between sites and years. In the case of earthworms, the weight of cocoons can be used, in broad terms, as indicator of the physiological state of adults. Cocoon weights of Dendrobaena octaedra were found to be constant between years and sites. In addition, annual production of cocoons was found to vary in relation to moisture conditions. These parameters thus furnish valid means of comparing Test and Control at the level of species-specific reproductive physiology.

3. System-level processes: to a limited degree, we have been monitoring litter input and turnover characteristics of Test and Control sites. Timing and amounts of litterfall have not differed between sites over the past four years, and turnover rates of forest floor litter have been estimated at approx. one year in both sites. There is, however, some error inherent in sampling forest floor litter; more specific estimates of decomposition were obtained by documenting mass loss of maple leaves of known initial

weight. Detailed data on breakdown of individual leaves showed that decomposition proceeded at the same rate in Test and Control. Similarly, litter confined in large-mesh net bags decomposed at comparable rates in both sites, with estimated turnover times of 1.3 and 1.4 years in Test and Control respectively. Despite some site-differences in the numbers and kinds of invertebrates present, process-level data on litter breakdown are thus good indicators of potential future disturbance.

I. REVIEW

The core objectives of our project consist of documenting arthropod and earthworm population dynamics in one Test and one Control site. Supportive environmental data, e.g., temperature and moisture, are obtained concurrently. A single process-oriented work element dealing with litter input and decomposition has emerged as feasible as well as useful for between-site comparison.

The main goal of our activities will be the detection of changes in any given parameter should they occur during operation of the ELF antenna. In the body of this report, we have begun to identify study elements with respect to their usefulness toward achieving that goal. In general, we have found that increasing the level of detail, e.g., by quantifying reproductive or recruitment parameters, enhances the sensitivity of our data base.

We have continued to pursue project objectives with essentially no change in methodology. Since 1986 can be considered as the last true pre-ELF year, and almost all data from 1984 through 1986 have been assembled, we are currently preparing several manuscripts spanning that time period. A list of publications to date and of manuscripts in preparation can be found in Appendix A.

II. ENVIRONMENTAL MONITORING

1. Precipitation

In Table 1, monthly precipitation totals of the past 4 years are compared to 30-year averages for the study area at large. Since rainfall events in Test and Control have generally been similar, only monthly data for Control are shown. Yearly totals for both sites (May through October) show that, with some variation, precipitation is comparable between sites (Table 1).

Over the past four years, major rainfall deficits occurred mainly in the first half of each field season. Noteable are the mid-summer drought of 1985, and the pronounced spring-summer drought of 1986 (Table 1). Total precipitation was, however, above average in 1985 due to increased autumn rains, and again in 1987, when rains were particularly ample in July and August (Fig. 1). The 1986 season was by far the driest experienced so far.

Table 1. Monthly precipitation in Control, 30-year means (Crystal Falls Weather Station), and totals for each field season in Test and Control, 1984 - 1987.

	Rainfall/ month, mm						Totals	
	May	June	July	Aug.	Sept.	Oct.	CONTROL	TEST
Year								
1984	27.1	76.6	76.3	129.0	124.4	-*	430.0*	464.8*
1985	47.4	78.4	53.8	137.4	174.1	78.6	569.7	524.5
1986	3.9	63.7	55.6	84.5	105.9	77.4	391.0	362.0
1987	68.9	69.3	152.7	124.5	49.7	64.7	529.8	535.3
30-yr mean	81.0	105.4	91.4	98.5	84.6	52.8	513.7	

* Note: in 1984, measurements were stopped in mid-October.

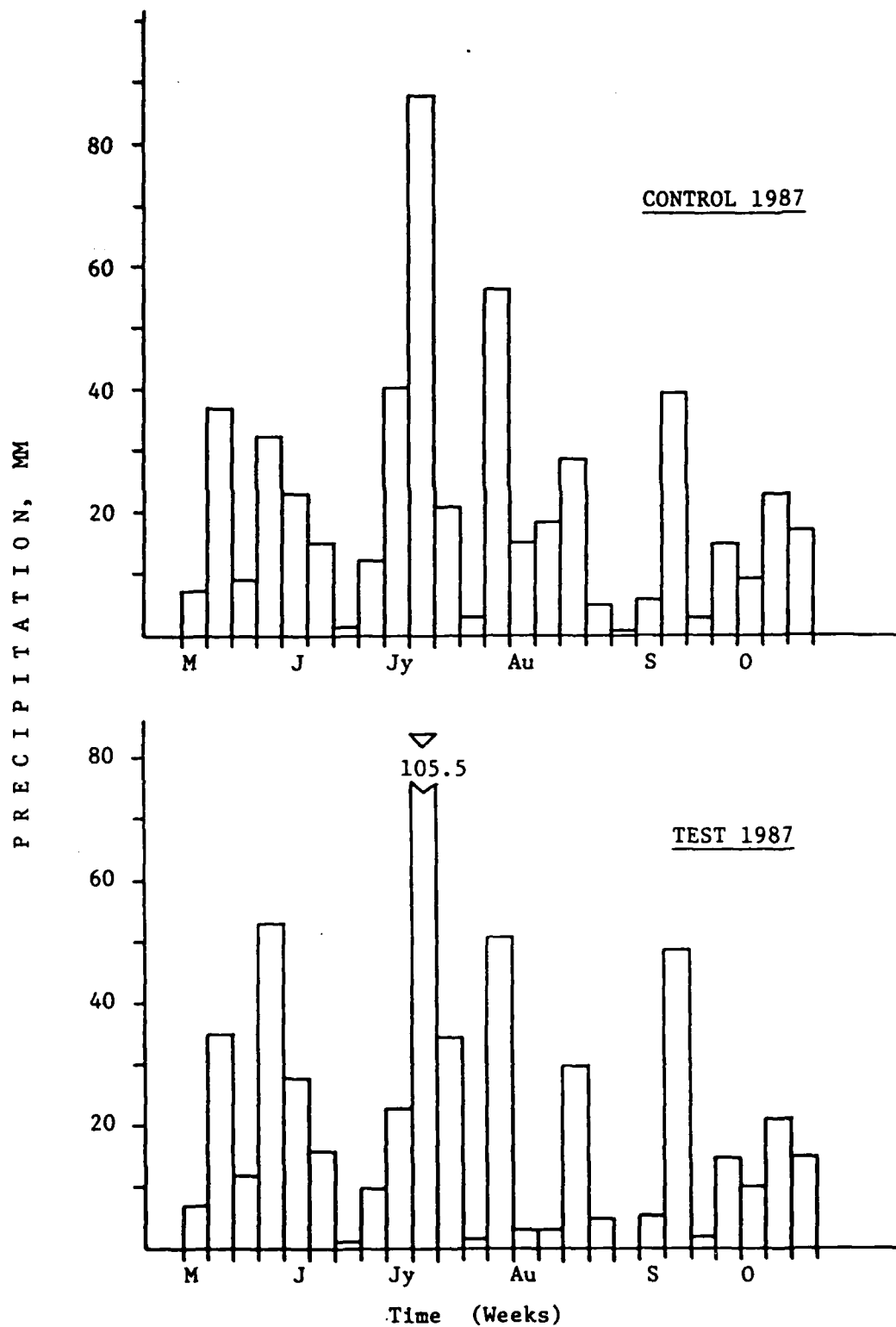


Fig. 1. Weekly precipitation totals for Test and Control sites, 1987.

2. Litter and soil moisture

Figure 2 illustrates bi-weekly litter moisture averages for the past four years. These point estimates generally reflect rainfall patterns, e.g., the prolonged spring drought of 1986, and the more frequent rains of 1987. Yearly means (Table 2) did not differ between sites. ANOVA of each year's data did not show a site effect for 1986 and 1987 ($P > 0.1$); neither were there significant differences in 1984 if the single discrepant date in July was disregarded (caused by an isolated rainfall event). Although sites were a significant source of variation in 1985, differences are likely to be of no biological significance, given the general concurrence of litter moisture fluctuations in Test and Control (Fig. 2).

Average yearly moisture estimates for A and B horizons are listed in Table 2. While B horizon moisture is essentially the same in both sites, the A horizon in Control has a greater tendency to retain moisture than that in Test. Bi-weekly estimates of A moisture (Fig. 3) again reflect rainfall patterns: a single brief decline in late July 1984; a mid-summer drought, followed by rapid re-hydration due to above-average August rains in 1985; a rapid moisture decline in early 1986, persisting into September; in 1987, with rainfall ample but unevenly distributed (Fig. 1), late June and early September were marked by brief moisture declines in the A horizon.

Not surprisingly, ANOVA of bi-weekly moisture data showed a significant site effect ($P < 0.01$) for all four years. Test and Control clearly differ in the moisture-retention capacity of their A horizons.

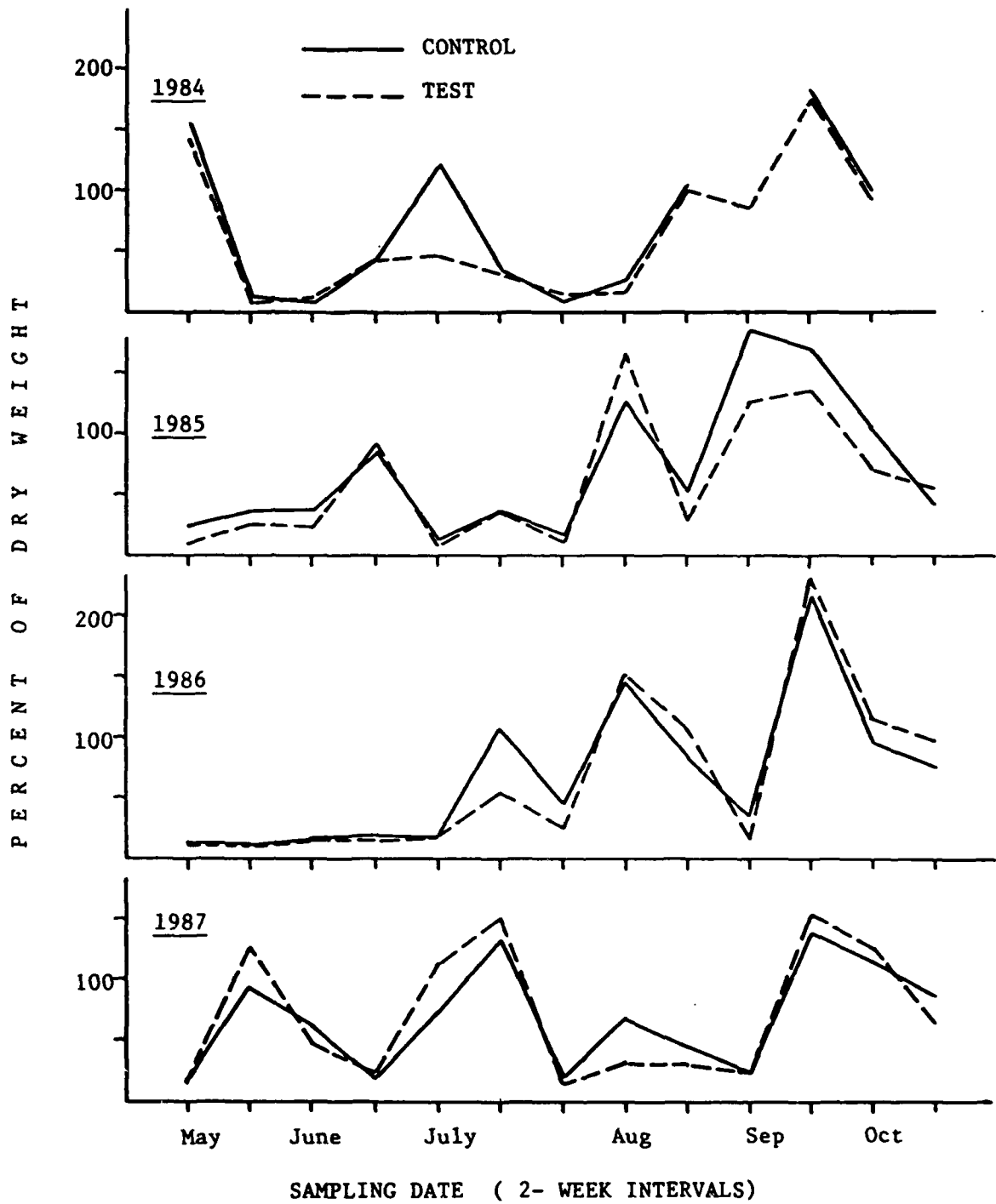


Figure 2. Average litter moisture, in percent of dry weight, in Test and Control, 1984 through 1987 (N = 20/ date / site).

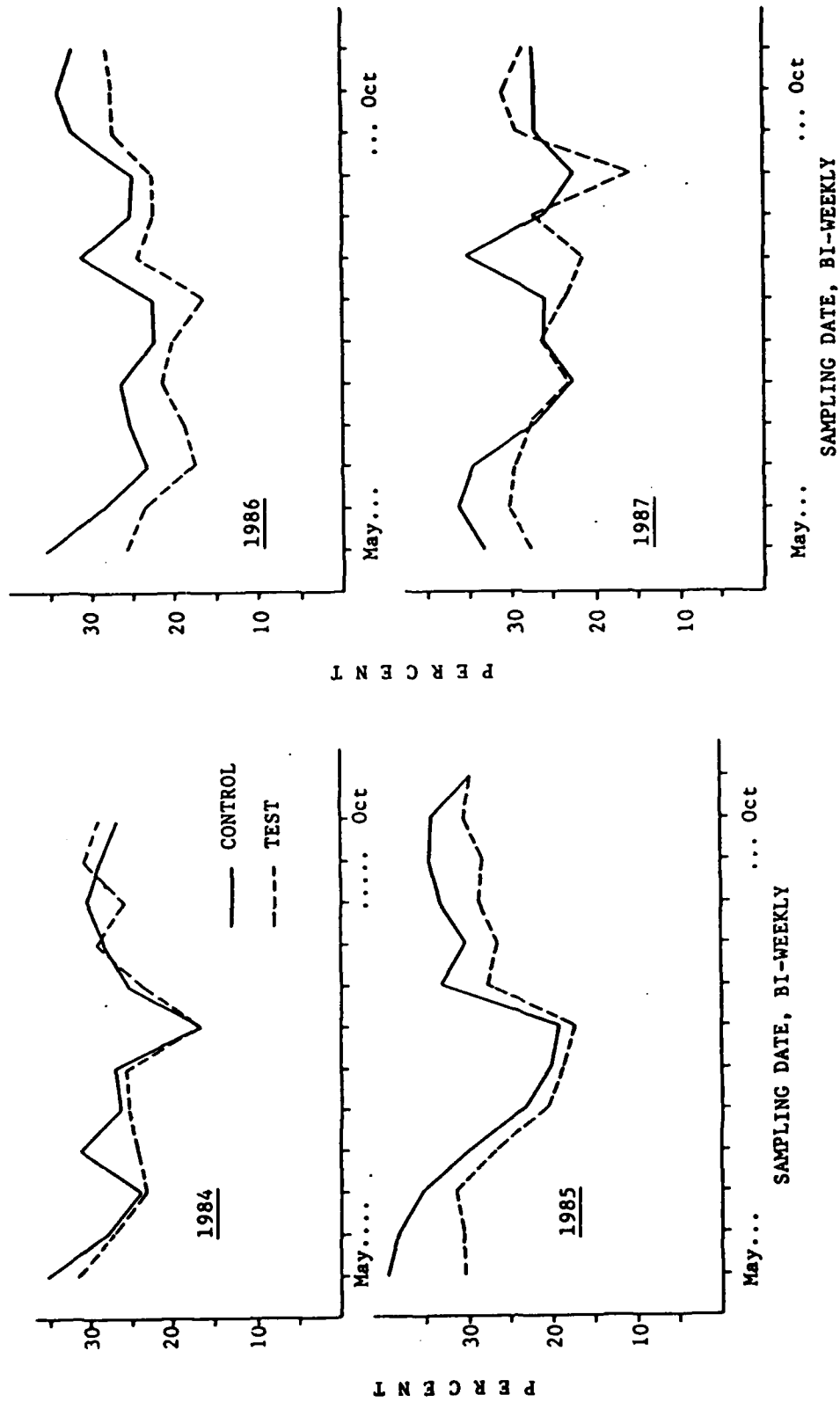


Fig. 3. Average moisture of A horizon, in percent of dry weight, in Test and Control, 1984 - 1987 (N = 20/ date/ site).

Table 2. Average yearly moisture levels of litter, A and B horizons, in percent of dry weight (means \pm 95% CL); for 1984, N = 240 samples, for 1985 - 1987, N = 260 samples/ site / depth.

	M e a n s \pm 95% C L			
	1984	1985	1986	1987
LITTER				
Test	64.01 \pm 7.34	61.06 \pm 6.70	67.17 \pm 8.38	70.05 \pm 6.85
Control	74.74 \pm 8.40	73.02 \pm 7.71	69.41 \pm 7.67	69.14 \pm 5.56
A HORIZON				
Test	25.94 \pm 0.88	26.75 \pm 0.95	22.80 \pm 0.78	26.17 \pm 0.85
Control	27.45 \pm 1.03	30.84 \pm 1.05	28.14 \pm 1.05	28.75 \pm 1.07
B HORIZON				
Test	15.14 \pm 0.55	15.85 \pm 0.61	12.69 \pm 0.44	14.52 \pm 0.47
Control	14.44 \pm 0.53	15.49 \pm 0.61	13.19 \pm 0.50	14.04 \pm 0.41

3. Temperature

3.1. Air temperature

Air temperatures have been essentially equal in Test and Control, daily means differing by $\leq 1.0^{\circ}\text{C}$ during any one season. In order to illustrate seasonal temperature profiles of the study area, weekly averages were derived from Omnidata records obtained by hygrothermographs above the forest floor (Fig. 4). With the exception of October, temperatures in 1987 were frequently higher than in previous years; in June in particular, high temperatures and below-average rainfall contributed to reduced soil moisture levels (Fig. 3).

3.2. Soil temperature

Initial sensor calibration in the laboratory did not show consistent

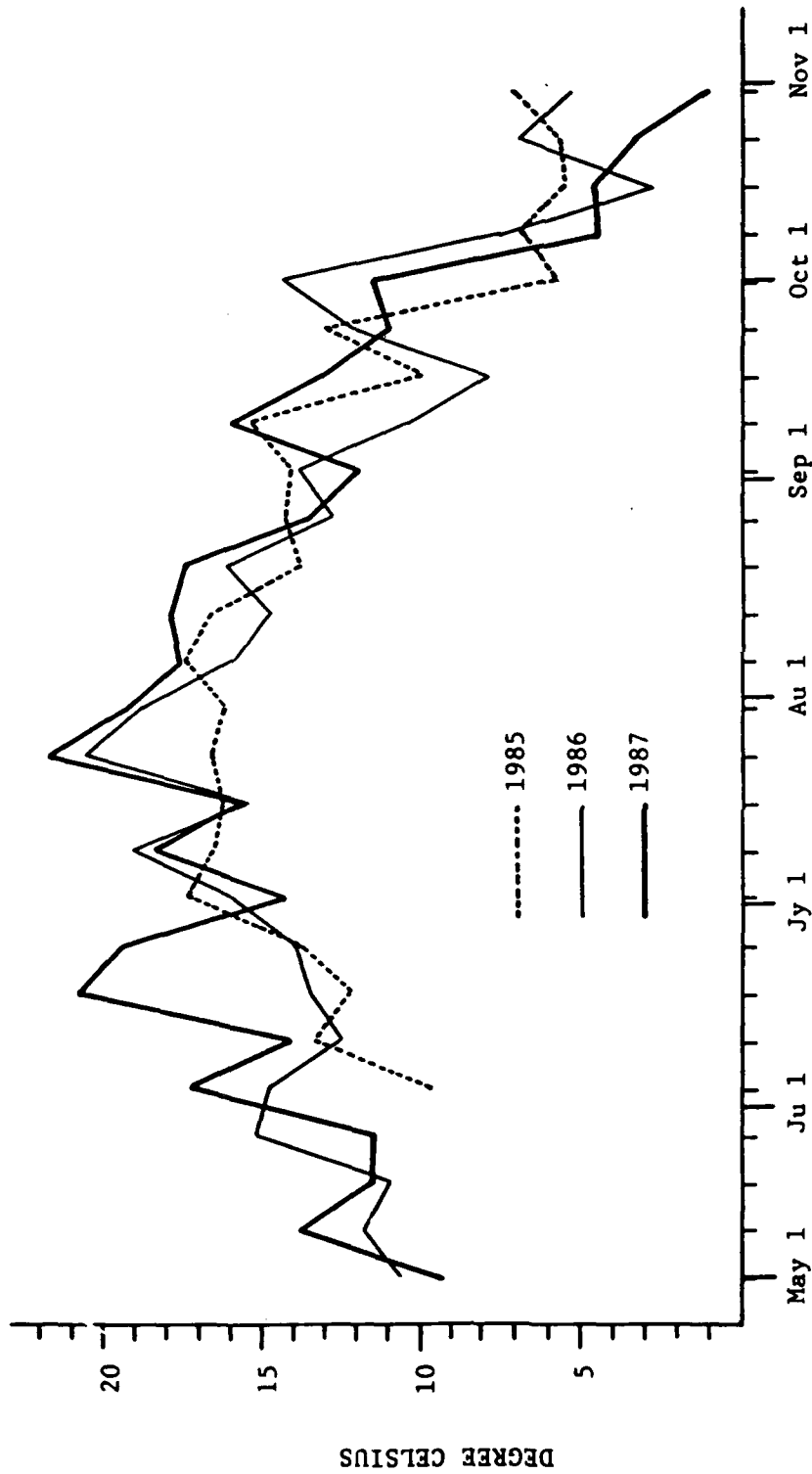


Fig. 4. Average weekly air temperature (Control), May 1 to October 30, derived from daily means of Omnidata readings at hourly intervals.

linear differences between those used in Test and those installed in Control. However, apparent site differences in soil temperature observed in 1985-86 led us to perform additional in-situ calibration via YSI telethermometer in 1987. These readings could then be compared with Omnidata logged temperatures at the same time of day. YSI measurements were taken in the immediate vicinity of buried sensors, as well as at random locations over each site.

The following conclusions were reached:

a) YSI measurements taken randomly over each site yielded means and ranges consistent with those obtained in the vicinity of buried sensors (means differed by ≤ 0.1 °C); i.e., data logged in a single central location are representative of the site in general.

b) Test and Control soil temperatures (via YSI) did not differ to any significant degree, although they tended to be slightly higher in Control (Table 3).

c) Omnidata readouts differed from YSI measurements in both sites and at both 5 cm and 15 cm depths (Table 3). Discrepancies were consistently larger in Test than in Control, particularly at 15 cm depth. Control temperatures at 5 cm depth were closest to YSI measurements; since data loggers record only in 0.5 °C increments, differences of approximately ± 0.5 °C have no significance.

Biologically, temperatures at 5 cm depth are the most important, since the A horizon is the preferred stratum of activity for lumbricids as well as arthropods. We propose to use Control temperatures from that horizon in faunal data analyses for both sites, knowing, however, that they may differ from actual temperatures by ± 1 °C in either site. Even with further investment in Datapods, sensors, and effort, we doubt that the accuracy and reliability of these measurements can be consistently improved. In-situ temperature checks will be repeated throughout the 1988 season.

Table 3. Soil temperatures at 5 cm and 15 cm depth, as measured by YSI telethermometer (N = 10/ date / depth) and by permanently installed Omnidata sensors.

	YSI Mean \pm 95% CL		Omnidata mean		Omnidata-YSI	
	Test	Control	Test	Con	Test	Con
5 cm depth:						
August 29	-	15.6 \pm 0.27	-	15.0	-	-0.6
September 1	12.3 \pm 0.08	12.3 \pm 0.19	14.0	13.0	+1.7	+0.7
September 2	12.6 \pm 0.33	12.6 \pm 0.26	14.0	13.0	+1.4	+0.4
October 4	9.8 \pm 0.17	10.2 \pm 0.20	10.5	10.5	+0.7	+0.3
October 25	4.4 \pm 0.18	5.2 \pm 0.18	5.5	5.0	+1.1	-0.2
15 cm depth:						
August 29	-	14.1 \pm 0.12	-	15.5	-	+1.4
September 1	12.3 \pm 0.11	12.3 \pm 0.24	15.0	13.5	+2.7	+1.2
September 2	12.0 \pm 0.10	12.2 \pm 0.39	14.5	13.5	+2.5	+1.3
October 4	8.5 \pm 0.16	9.3 \pm 0.29	11.0	10.5	+2.5	+1.2
October 25	4.3 \pm 0.51	5.1 \pm 0.18	7.0	6.0	+2.7	+0.9

III. SOIL AND LITTER ARTHROPODA

In 1986 and 1987, soil cores were sugar-floated following Tullgren extraction in order to obtain more accurate population estimates, particularly for Onychiuridae.

At this time, all 1986 and 1987 samples of litter arthropods, and all soil cores (Tullgren as well as floatation samples) have been rough-sorted. Identification of Collembola has been completed through 1986; 1987 Collembola data are expected to be completed at the beginning of the current (1988) field season. Determination of mites is still lagging. We have found that sugar-floatation occasionally yields significant numbers of mites, and will withhold 1986 and 1987 data until these specimens have been processed. Based on 1986 data, we will then assess whether, with respect to the few species of interest, the additional effort involved in handling "floatation mites" is warranted.

However, we have doubled the manpower allocated to mite determinations. At the same time, the effort involved was reduced by selectively sorting out only those species which have proven of interest to this project. We expect to have a full pre-ELF data base assembled and analyzed by late summer of 1988, at which time we will have caught up to samples obtained in the current field season.

1. Extraction efficiency for Collembola (1986)

A small number of non-onychiurid Collembola were recovered by floatation, but the majority of specimens were onychiurids, as expected. Over the whole year (1986), heat extraction efficiency for this family was estimated at 25% for Control and 32% for Test. In both sites, efficiency was lowest for

Tullbergia clavata and highest for T. iowensis (Table 4).

Percent of the most abundant species (T. mala and T. granulata) extracted in Tullgren funnels varied greatly from date to date, tending to be lower during the second half of the season (Fig. 5). No consistent relationships with soil moisture or temperature emerged. Most likely, consistency and structure of each individual sample caused the observed variability.

Table 4. Extraction efficiency of Tullgren funnels for common Onychiuridae (1986), in percent of total number of individuals obtained by [heat extraction + subsequent floatation].

	T E S T		C O N T R O L	
	Total indiv.	% heat-extr.	Total indiv.	% heat-extr.
<u>Onychiurus similis</u>	70	18.6	155	20.1
<u>Tullbergia clavata</u>	194	17.6	175	13.7
<u>Tullbergia mala</u>	609	27.3	4646	23.5
<u>Tullbergia granulata</u>	923	29.2	1471	28.4
<u>Tullbergia iowensis</u>	403	54.5	151	43.0
Totals	2199		6598	
Heat-extracted totals (%)	705	(32.1)	1628	(24.7)

2. Collembolan community structure

We calculated and tested diversity indices (h) after Hutcheson (1970) for the litter and soil subcommunities, based on total number of individuals per species. Combined [litter + soil] estimates were based on summed yearly densities per species. Indices are listed in Table 5.

In general, diversity of the litter subcommunities decreased in both sites

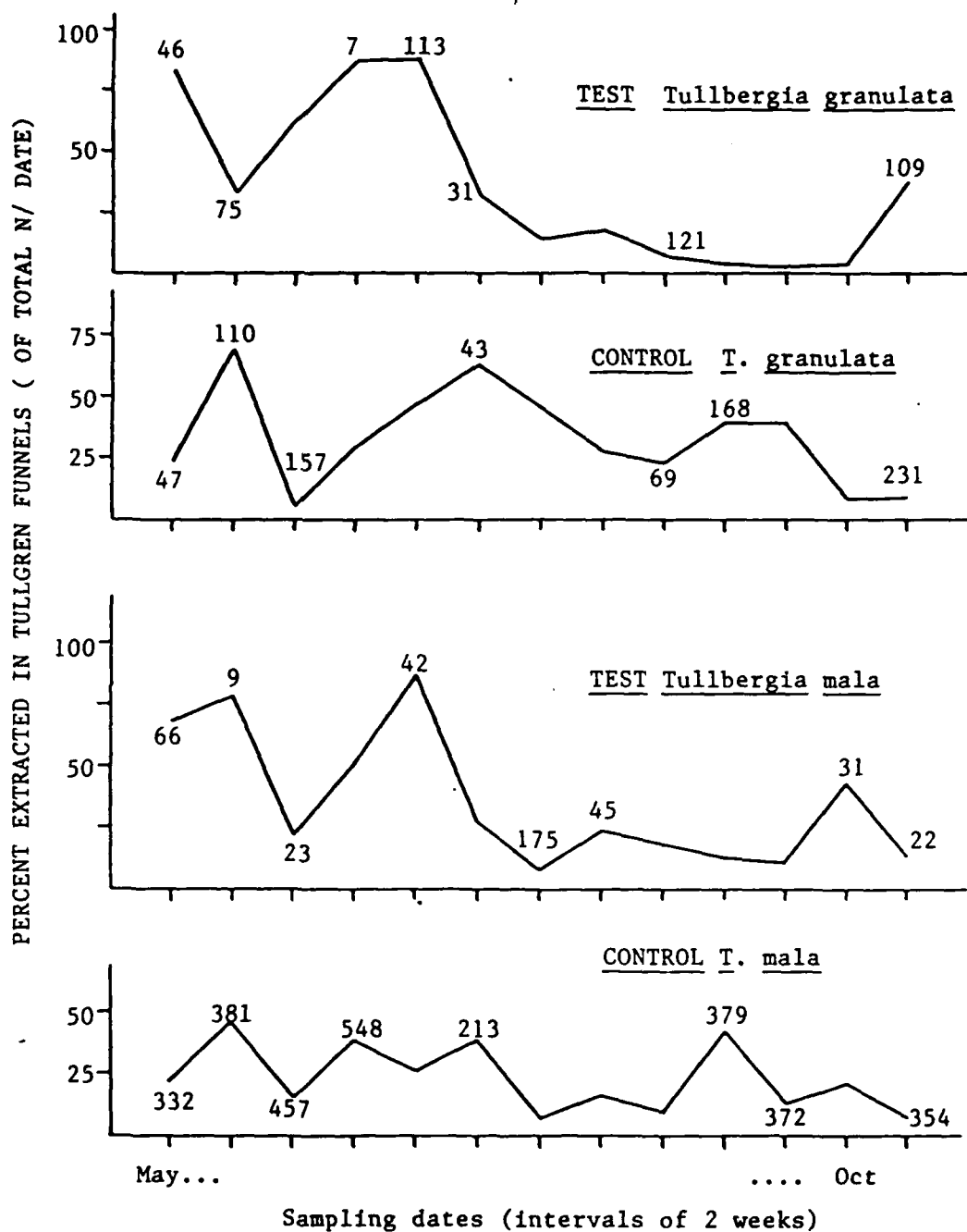


Fig. 5. Efficiency of heat extraction for two dominant onychiurid species in Test and Control, in percent of total number [heat extraction + floatation]. Total number of individuals indicated for randomly selected dates.

over the three years, that of the soil subcommunities increased in both sites. Virtually all between-site and between-year (within-site) comparisons showed significant differences at $P < 0.001$. Particularly discrepant were those indices which included Onychiuridae (Table 5). Diversity in Control was now much reduced due to the overwhelming dominance of the family in the soil species assemblage.

Table 5. Diversity indices for soil and litter Collembola in Test and Control, 1984 - 1986.

	Diversity index (h) *		
	1984	1985	1986
Test litter	2.354	1.830	1.905
Control litter	1.955	1.309	1.114
Test soil	2.047	2.365	2.397
Control soil	2.098	2.004	2.182
Test soil + Onych.	-	-	2.386
Control soil + Onych.	-	-	1.495
Test litter + soil	2.158	2.343	2.398
Control litter + soil	2.162	1.952	1.277

* Note: $h = \sum_{i=1}^S (n_i/n) \ln(n_i/n)$

Community-level comparisons are difficult to interpret. We may expect some random fluctuation from year to year as uncommon species appear and vanish from samples, and as major fluctuations in abundant species occur. Since data needed for diversity measures are acquired as a matter of routine, we will continue to quantify these measures in order to monitor potential long-term trends.

3. Collembolan abundance

3.1. Family-level comparisons

A first assessment of between-year fluctuations can be obtained at the family level. Summed litter and soil density estimates (Table 6) show increased numbers of Hypogastruridae and Isotomidae over the three-year span, in both sites. Entomobryidae decreased slightly in Control, but increased in Test. Sminthurid numbers fluctuated in Test, but increased in Control. A first estimate for Onychiuridae, based on total number obtained by Tullgren extraction and floatation, clearly demonstrates the high dominance of that family, particularly in Control soil (Table 6).

Table 6. Mean annual density/m² (estimates from leaf litter and soil samples combined) for collembolan families in Test and Control, 1984-1986.

	TEST N/m ²			CONTROL N/m ²		
	1984	1985	1986	1984	1985	1986
Sminthuridae	267	471	298	247	374	459
Entomobryidae	821	1065	1417	217	190	172
Isotomidae	1537	2261	2586	2727	3938	3571
Hypogastruridae	34	214	526	254	387	726
Neelidae	258	158	18	225	178	293
Onychiuridae	-	-	8531	-	-	25592

3.2. Densities of common species

Between-year changes in abundance at the family level (Table 6) were strongly influenced by the dominant species. In soil, only I. notabilis (of all non-onychiurids) occurred in high enough frequency to allow site comparison. In both sites, mean yearly densities of the species increased

over the three-year span (Table 7). Population estimates for the most abundant Onychiuridae are included in Table 7, although only for 1986 (when floatation of samples after Tullgren extraction yielded more accurate estimates).

Populations in leaf litter (Table 8), which span a wider range of frequently extracted species, also reflected the family-level estimates of Table 6: S. henshawi, relatively stable over the years; I. notabilis, increasing in both sites, peaking in 1985; and for both T. flavescens and O. hexfasciata, increases in Test and decreases in Control (Table 8).

Table 7. Average yearly abundance \pm SE of dominant soil Collembola in Test and Control. N = 120 in 1984, 130 in 1985-86.

	N / m ² \pm SE		
	1984	1985	1986
<u>I. notabilis</u> , TEST	1008 \pm 167	1400 \pm 284	1573 \pm 422
<u>I. notabilis</u> , CONTROL	1338 \pm 198	2061 \pm 260	1985 \pm 297
<u>T. granulata</u> , TEST	-	-	3550 \pm 930
<u>T. granulata</u> , CONTROL	-	-	5658 \pm 1253
<u>T. mala</u> , TEST	-	-	2342 \pm 795
<u>T. mala</u> , CONTROL	-	-	17869 \pm 2834

Table 8. Average yearly abundance \pm SE of litter-dwelling Collembola common to Test and Control. N = 120 in 1984, N = 130 in 1985-86.

	N / m ² \pm SE		
	1984	1985	1986
<u>S. henshawi</u> , TEST	35.7 \pm 6.1	39.3 \pm 5.0	31.5 \pm 3.6
<u>S. henshawi</u> , CONTROL	56.7 \pm 7.8	88.9 \pm 16.1	65.0 \pm 6.9
<u>I. notabilis</u> , TEST	118.4 \pm 21.0	283.6 \pm 35.8	209.1 \pm 26.6
<u>I. notabilis</u> , CONTROL	290.8 \pm 39.7	882.5 \pm 85.7	704.5 \pm 191.5
<u>T. flavescens</u> , TEST	55.9 \pm 10.9	82.7 \pm 10.2	75.7 \pm 10.9
<u>T. flavescens</u> , CONTROL	17.3 \pm 3.0	16.1 \pm 2.5	< 5.0
<u>O. hexfasciata</u> , TEST	15.6 \pm 2.7	22.2 \pm 4.1	41.5 \pm 5.7
<u>O. hexfasciata</u> , CONTROL	11.2 \pm 3.0	< 10.0	< 5.0

The array of species postulated as potential test cases in early project years now becomes narrowed to fewer than expected. Entomobryids, mainly litter inhabitants, decreased in Control to the point where between-site testing becomes impossible (Table 8). Sminthurinus henshawi in litter, and Isotoma notabilis in litter and soil, remain as candidates for long-term analysis. Two onychiurids, numerical dominants in both sites, potentially qualify in terms of large available numbers (Table 7).

3.3. Analysis of seasonal densities

1. Litter Collembola

Since litter-dwelling entomobryids decreased considerably in 1986 in Control, 1984-85 data for T. flavescens and O. hexfasciata were subjected to ANOVA of differences between means/date. Site effects were highly significant ($P < 0.001$) in both years.

For the litter-dwelling subpopulation of I. notabilis as well as for S. henshawi, site effects in 1984, 85 and 86 were equally significant at

$P < 0.001$. We illustrated population fluctuations of S. henshawi in Fig. 6, and of I. notabilis in Fig. 7; these Figures support the conclusion that data for litter species, taken per se (without potentially powerful covariates) may be too variable for between-site analysis.

Between-year differences in abundances (e.g., S. henshawi, Fig. 8) suggest two or three main sources of variation:

- a) density-dependency, i.e., low abundance in the first half of the season, generally larger numbers in the second half. Superimposed on a) are:
 - b) litter moisture on a given sampling date, and
 - c) litter mass of individual samples.

We have so far focused on analyzing the seasonal dynamics of I. notabilis, because data on population size structure have recently been completed (see sect. 3.4.). In order to identify potential covariates for ANOVA of abundances, a preliminary series of regressions on edaphic variables were performed:

- a) Regression of mean number of individuals in leaf litter on mean litter moisture ($N = 38$ dates over three years). In Control, 37% of observed variation was thus accounted for, in Test, however, only 13%. Without doubt, changing seasonal densities due to recruitment affected these results.

- b) Date-specific regressions (i.e., independent of seasonal density effects) of the number of individuals/ sample on sample moisture ($N = 10$ /date) showed no consistent relationships. At low litter moistures (say, $\leq 20\%$), most regressions were not significant ($P > 0.25$) due to low frequency of the species in all samples. At intermediate as well as high moistures, significance levels varied from $P < 0.005$ to $P > 0.25$. with no clear relationship between population distribution and litter moisture.

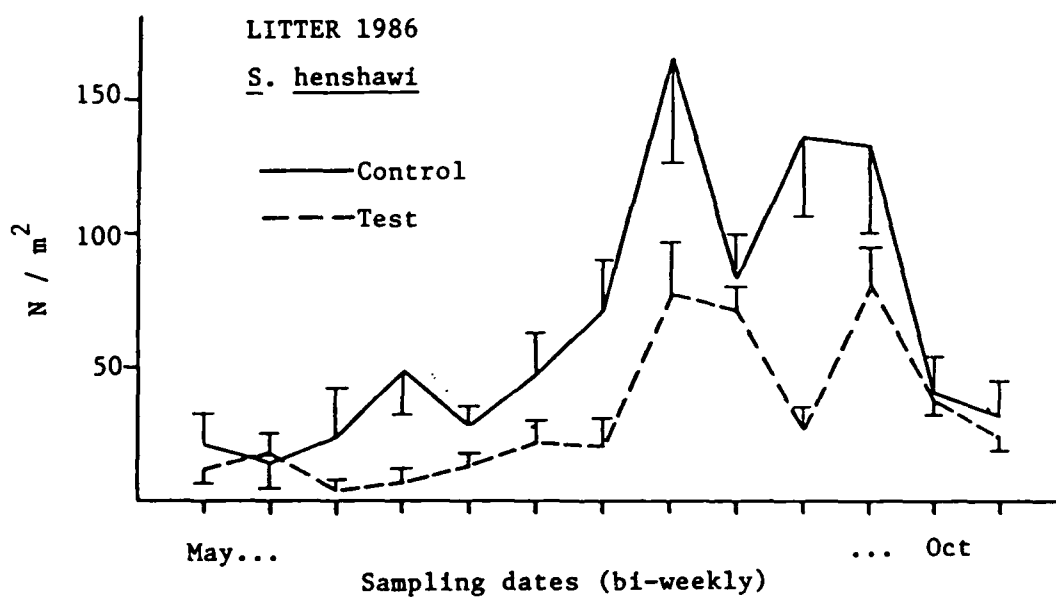


Fig. 6. Densities of Sminthurinus henshawi in leaf litter, 1986 (means \pm SE).

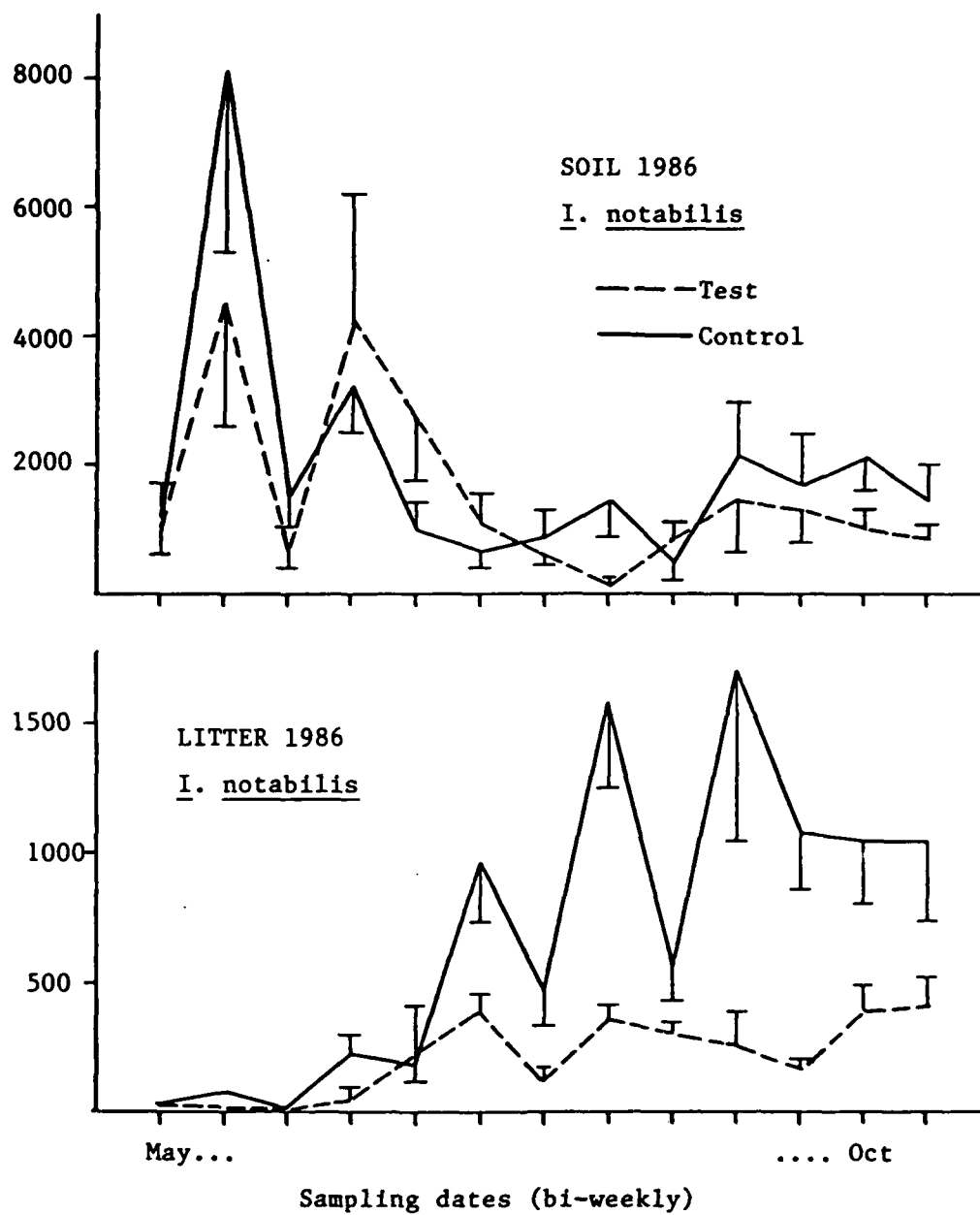


Fig. 7. Density (means \pm SE) of *Isotoma notabilis* in leaf litter and soil, 1986.

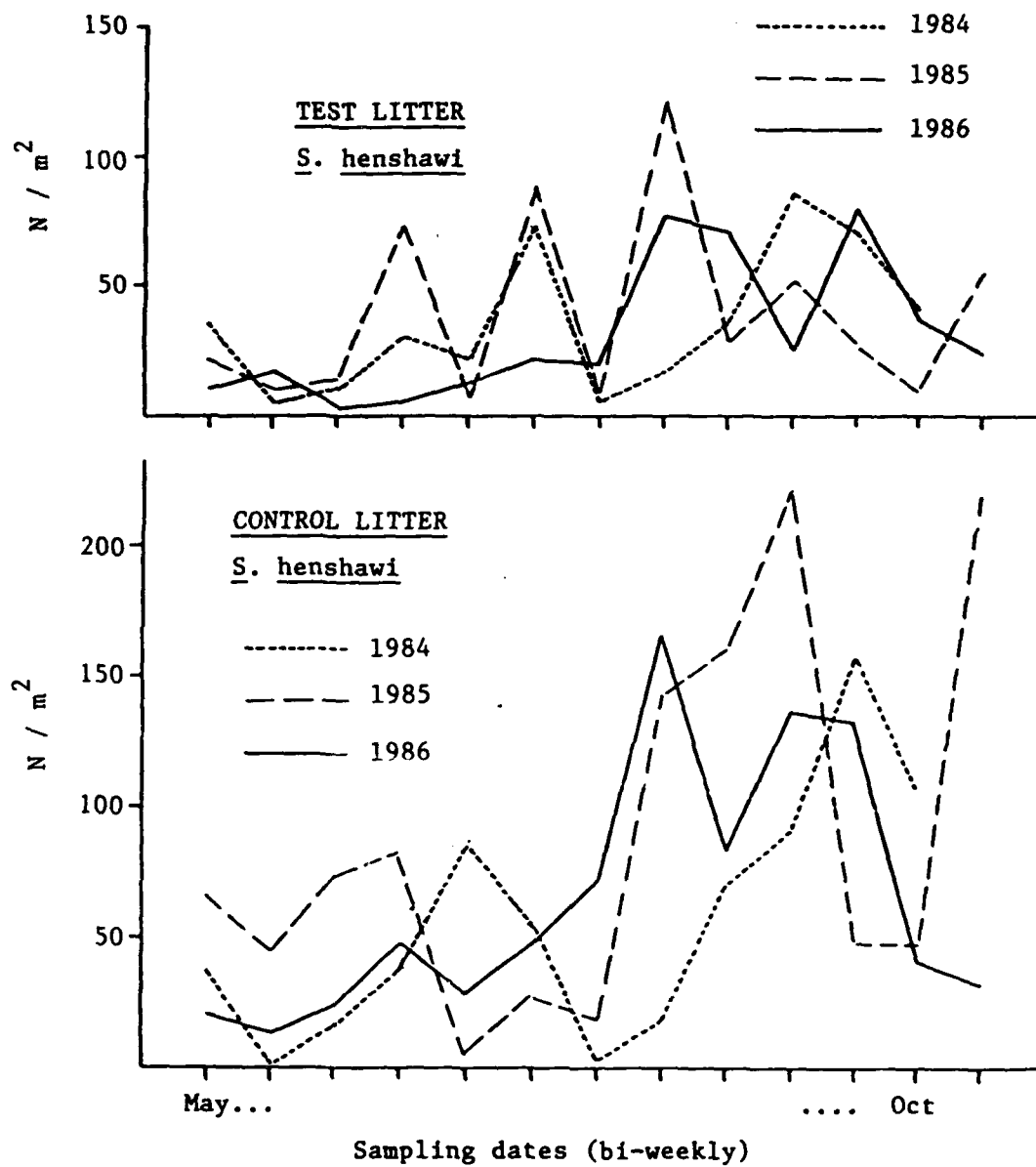


Fig. 8. Densities of *Sminthurinus henshawi* (means) in three consecutive years in leaf litter.

c) Date-specific regressions of number of I. notabilis on individual litter sample mass ($N = 10/\text{date}$). In this case, very few regressions were significant, at $P < 0.05$, on dates when litter moistures were low, but not extremely so.

Despite somewhat discouraging preliminary results, we are pursuing these analyses further. Test/Control abundances may be useful for this project if interactions between litter mass and moisture, as well as population structure, can be taken into account simultaneously.

ii. Soil Collembola

Efforts to rectify poor extraction efficiencies for Onychiuridae have resulted in much larger numbers of specimens and more accurate abundance estimates. However, even without analysis, it is now obvious that seasonal abundances not only vary drastically between sites, but populations are so highly aggregated that detection of changes may become meaningless.

Fig. 9 illustrates seasonal densities of T. mala and T. granulata. In this case, we have used 95% confidence limits of the means in order to emphasize the point made above. In some cases, e.g. T. granulata in Test, true population means may have values including zero, or close to zero.

At the species level, the one candidate left for site comparison is I. notabilis. Although these populations also show a high degree of aggregation, seasonal fluctuations have been relatively synchronous in Test and Control soils (Fig. 7 shows 1986 data).

Results of an initial ANOVA of differences between means ($\log y+1$, each year analysed separately) indicated significant site effects for 1985 ($P < 0.05$), but not for 1984 and 1986 ($P = 0.08$ and 0.09 respectively). Using the combined evidence of all three years, site effects were significant at $P = 0.0012$. A "combined evidence test" was used to check the validity

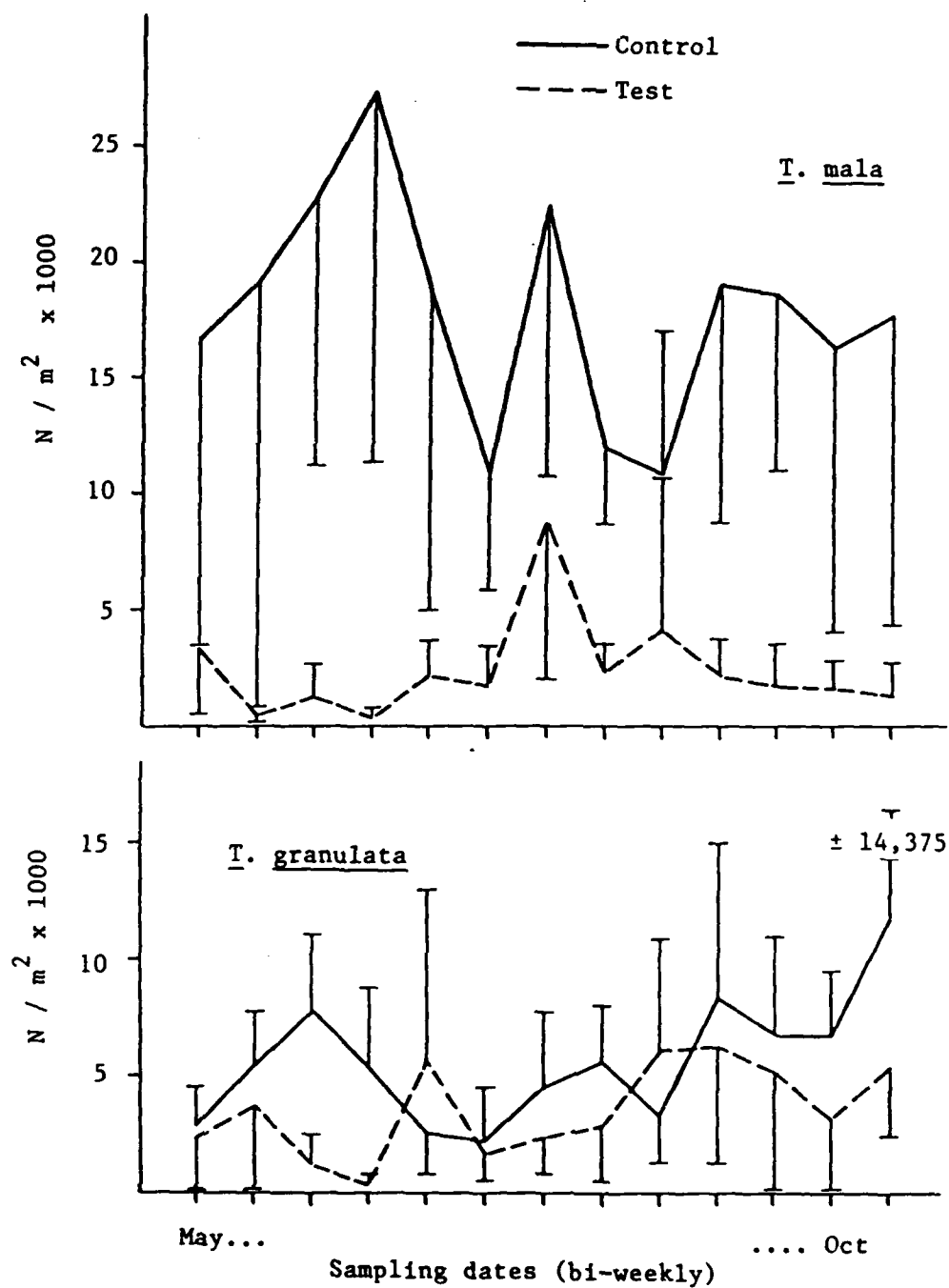


Fig. 9. Average seasonal densities \pm 95% CL of *Tullbergia mala* and *Tullbergia granulata* in Test and Control soil, 1986.

of this result, which was confirmed ($q = -2 \sum \ln \alpha_i$, $i = 1984-86$;
 $q = \text{Chi square}_6 = 18.647$, i.e., $0.005 > P > 0.001$).

These data suggest that differences between sites are marginal, and that density estimates may be used for site comparison if appropriate covariates are employed. Timing and extent of recruitment, for instance, can furnish a quantifiable basis for interpretation and analysis of seasonal abundances (and their relationship with edaphic factors in leaf litter as well as soil).

3.4. Population structure of *Isotoma notabilis*

Environmental factors can influence population estimates of litter and soil Collembola, particularly for species prone to migration (McBrayer et al. 1977; Wolters 1983; Joosse and Groen 1970; Hassall et al. 1986). Patterns of recruitment may also cause abundance fluctuations, since reproduction and hatching are generally determined by temperature (Huhta and Mikkonen 1982; Joosse 1969).

In view of the high between-site variability of arthropod densities, we looked for additional reference points useable in population analyses. Among Collembola, *I. notabilis* is one of the most common species in litter and soil of both sites, and we have begun assembling data on its population structure.

Body length measurements of *I. notabilis* have recently been completed for 1984 through 1986 (soil-extracted specimens from 1986 are being processed at this time). First instars, older immatures, and adults constitute the three developmental classes distinguished by external morphology. Specimens are measured by ocular micrometer, and size classes are recorded in increments of 0.125 mm. First instars were found to measure 0.25 - 0.35 mm; other juveniles

0.3625 - 0.525 mm; and adults 0.5 - approximately 0.95 mm in length. There is a narrow overlap between adults and large juveniles in the 0.5 - 0.525 mm range (Calandrino, thesis research),

At this time, data summaries are incomplete and analyses non-existent. Simply for the purpose of demonstrating the nature of this data base, however, we present a few preliminary Figures and comments below.

Total numbers of individuals in each size increment are graphed in Figures 10-11 (litter subpopulation) and Figures 12-13 (soil subpopulation), for 1984 and 1985. Analysis of frequency distributions, by lumped size classes as well as by developmental stage, is pending.

Abundance estimates for adults (Fig. 14), total juveniles (Fig. 15) and first instars alone (Fig. 16) were as variable as those for total individuals discussed earlier. It may well be that recruitment patterns differ between sites (Fig. 16, 1985) and between years (Fig. 16, 1984). In general, however, juvenile and adult abundances (e.g., 1985, Fig. 17) fluctuated relatively synchronously, decreases in particular being influenced by low litter moisture in early and late July (ref. Fig. 2).

Population structure, which is of intrinsic interest because it reflects recruitment and growth, may also furnish covariance data for density analyses. Until the spatial and temporal distribution of I. notabilis stages are quantified, we cannot predict what form this parameter will take in overall population analyses.

4. Acari

We have reported previously that Acari as a group exhibited a numerical decline from 1984 to 1985 in both sites. Furthermore, species shared between

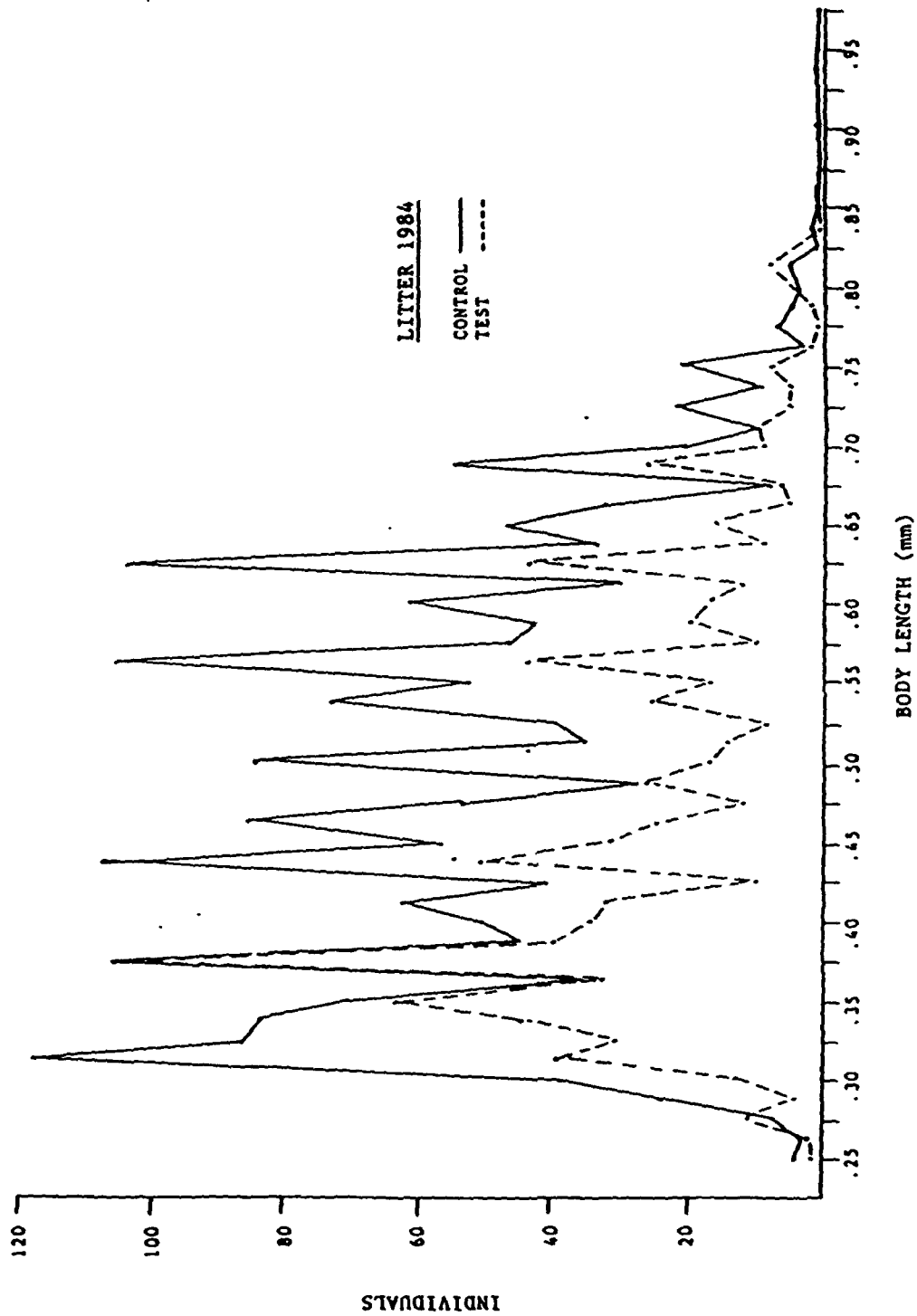


Fig. 10. Total numbers of Isotoma notabilis in each size class, extracted from leaf litter samples in 1984. (Calandrino, thesis research).

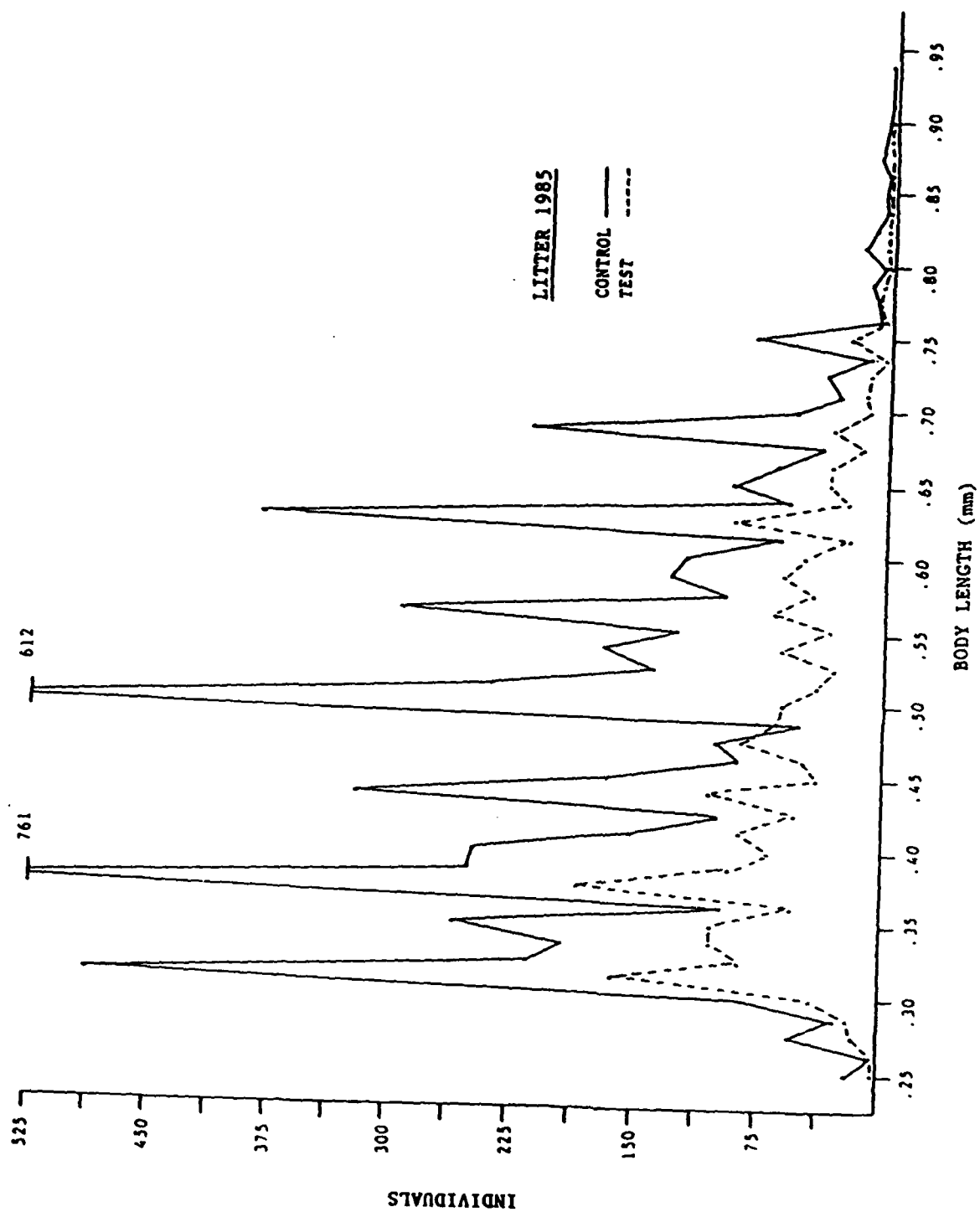


Fig. 11. Total numbers of Isotoma notabilis in each size class, extracted from leaf litter samples in 1985. (Calandrino, thesis research).

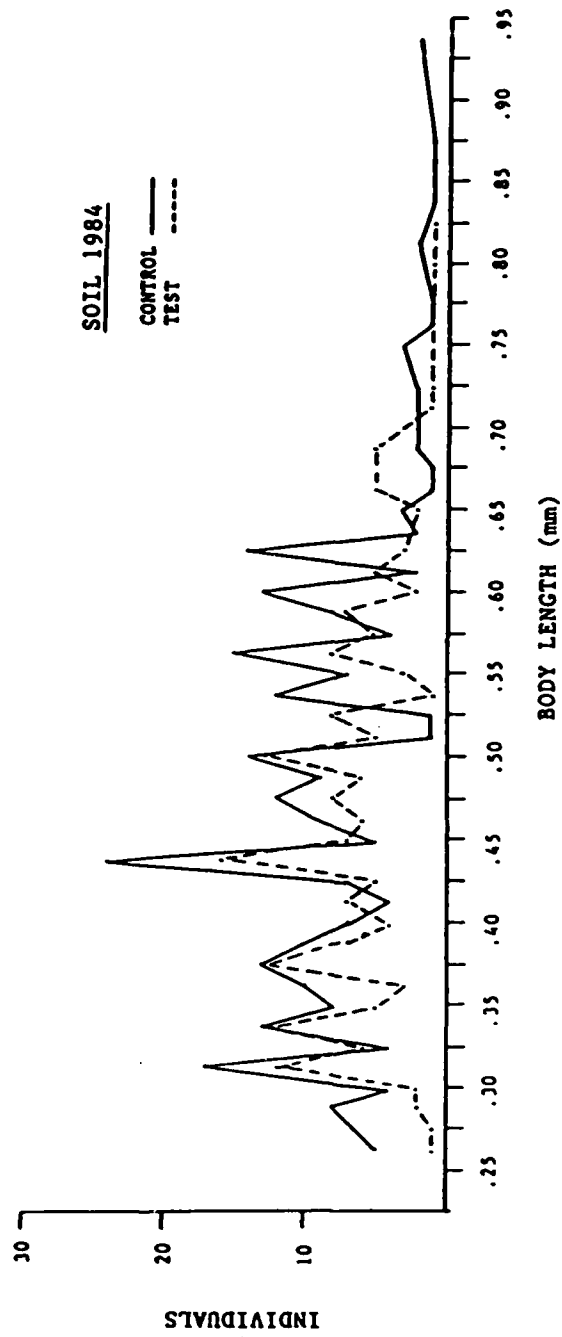


Fig. 12. Total numbers of Isotoma notabilis in each size class, extracted from soil core samples in 1984 (Calandrino, thesis research).

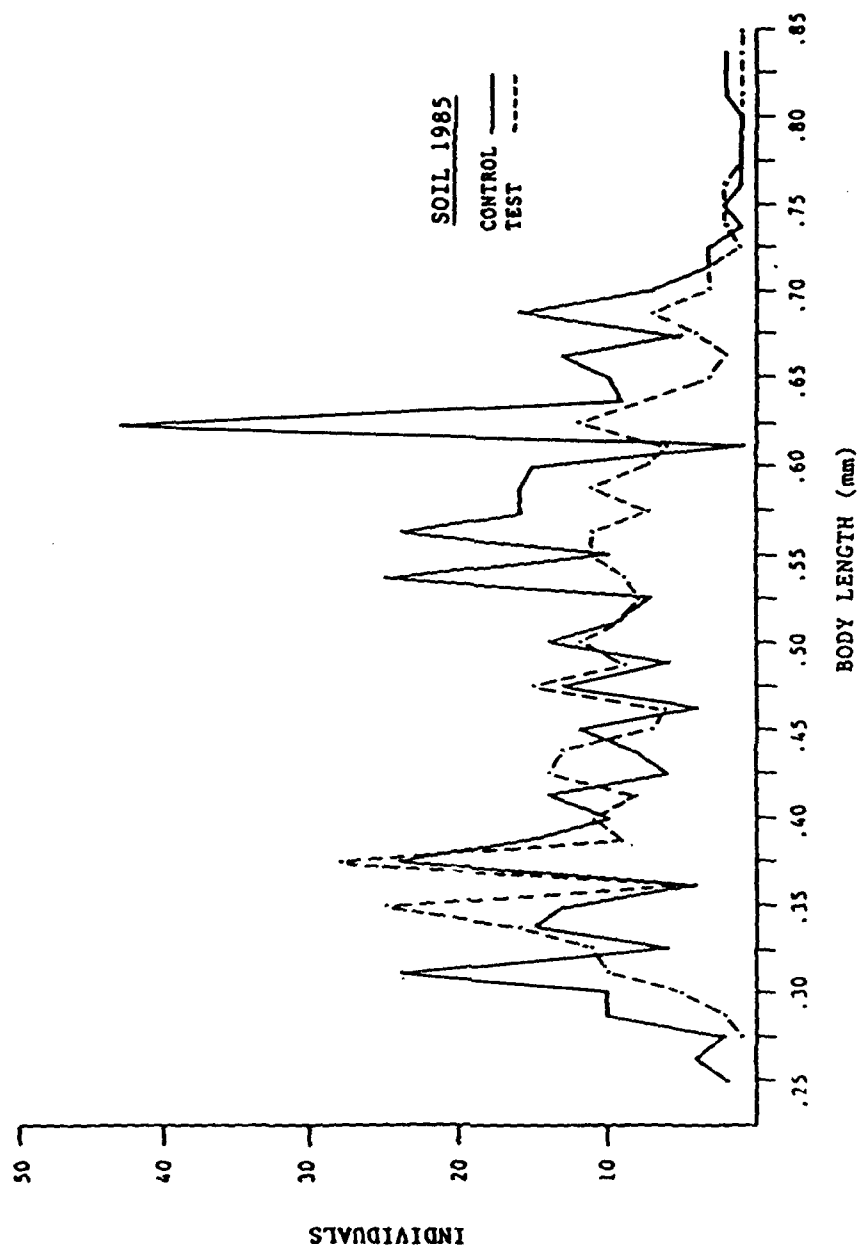


Fig. 13. Total number of *Isotoma notabilis* in each size class, extracted from soil core samples in 1985 (Calandrino, thesis research).

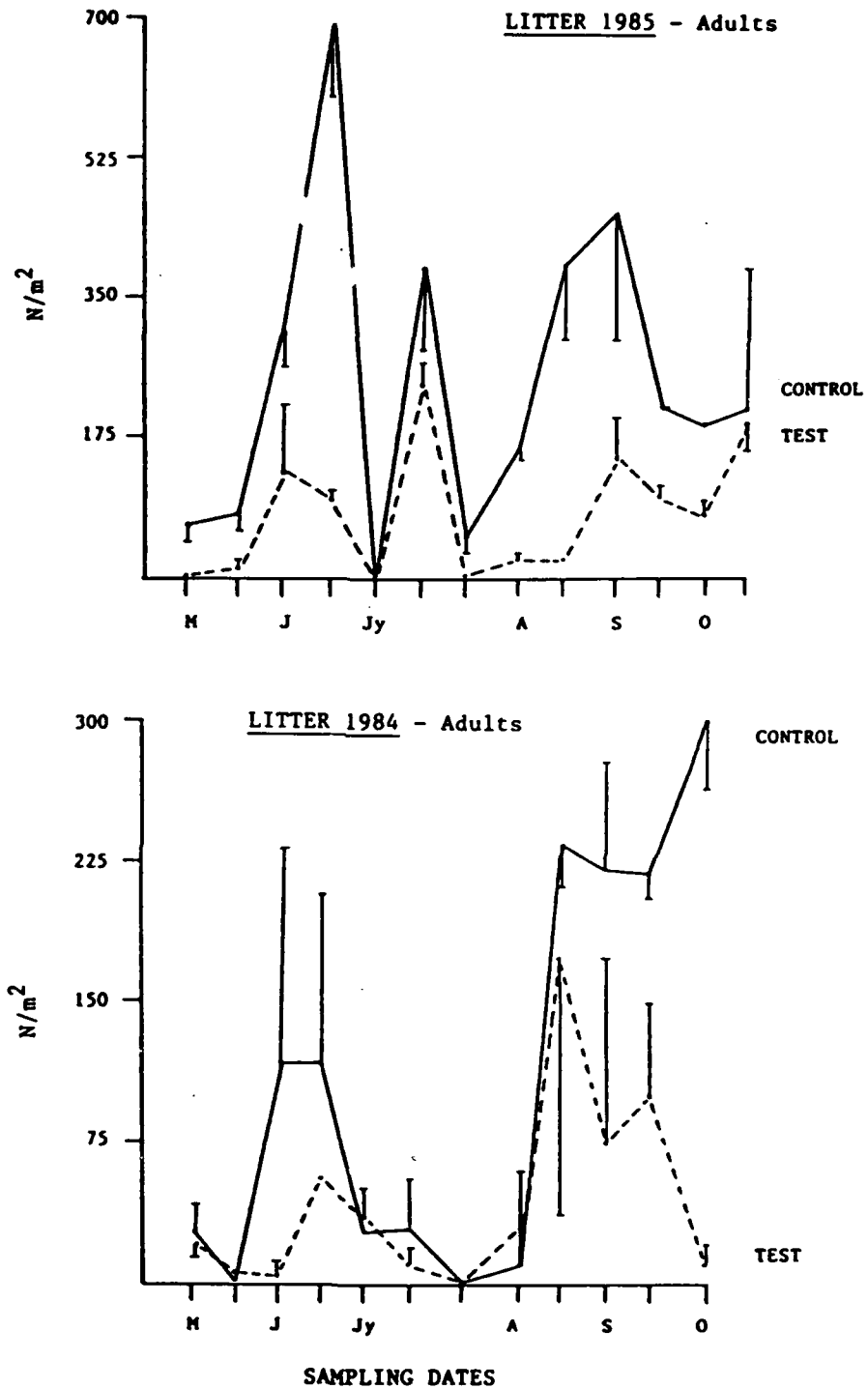


Fig. 14. Average densities \pm SE of *Isotoma notabilis* in leaf litter, 1984 and 1985, for adults. (Calandrino, thesis research).

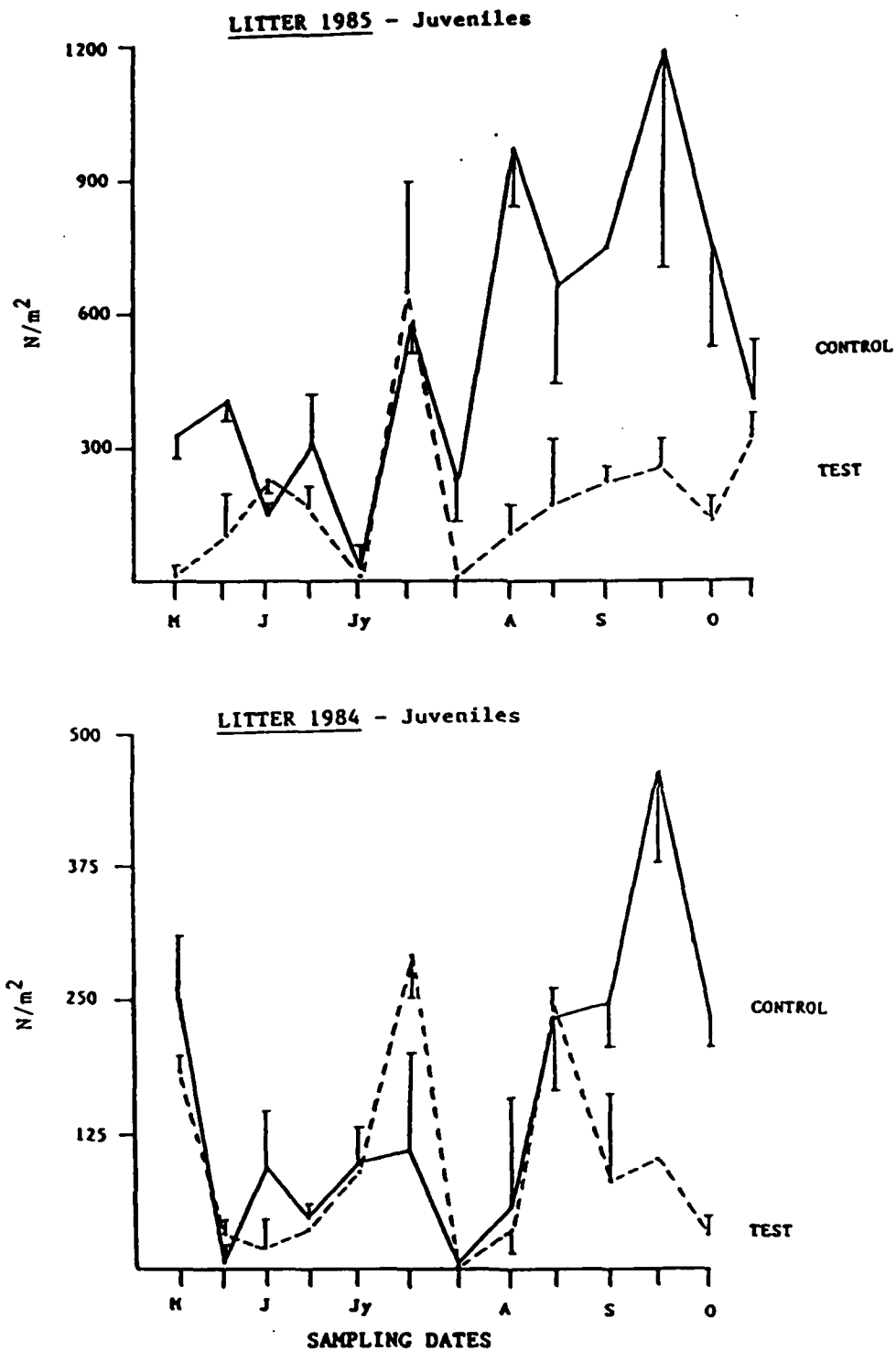


Fig. 15. Average densities of juvenile *Isotoma notabilis* (all instars) in leaf litter, 1984 and 1985 (means \pm SE) (Calandrino, thesis research).

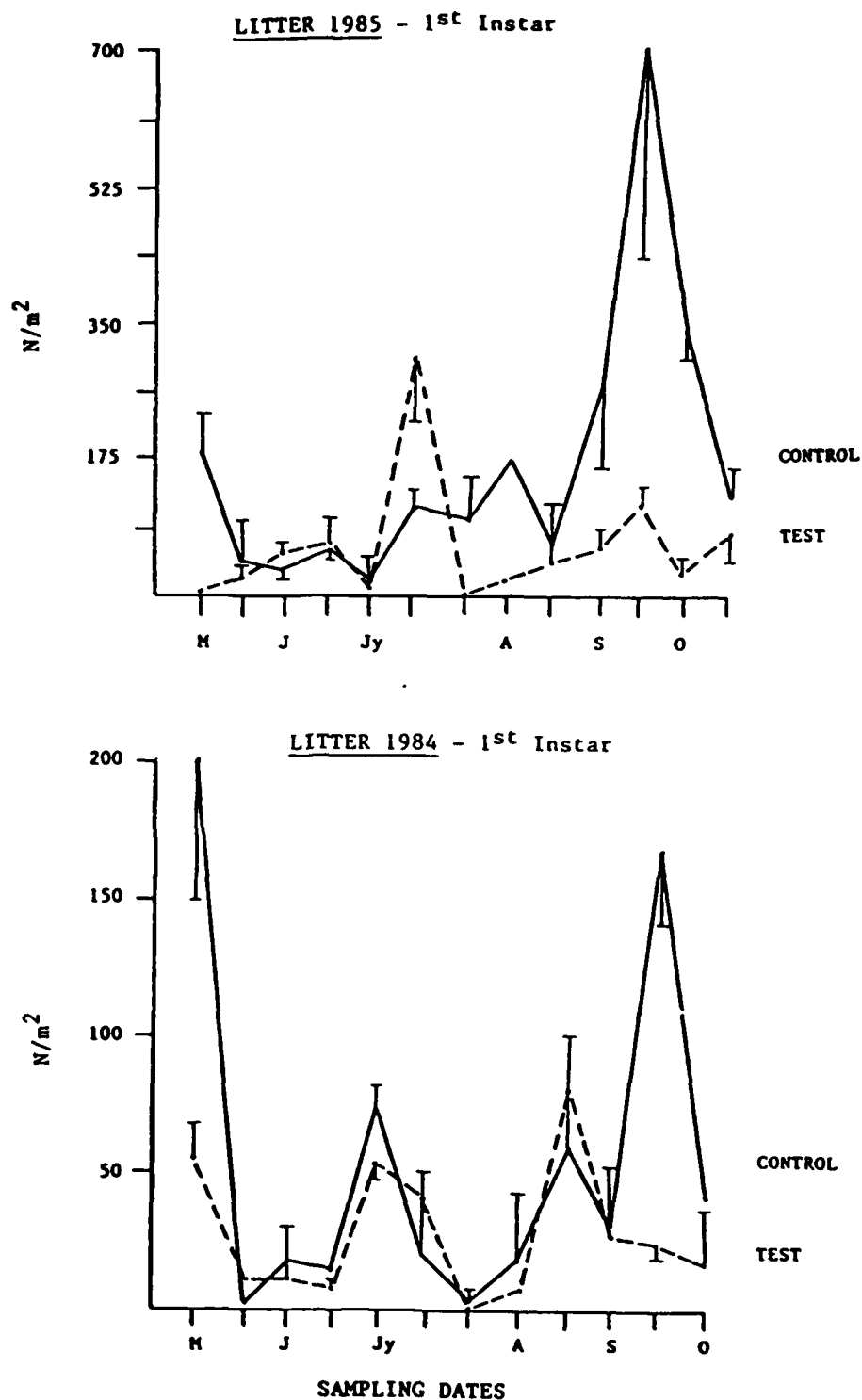


Fig. 16. Average densities \pm SE of first instar Isotoma notabilis in leaf litter, 1984 and 1985. (Calandrino, thesis research).

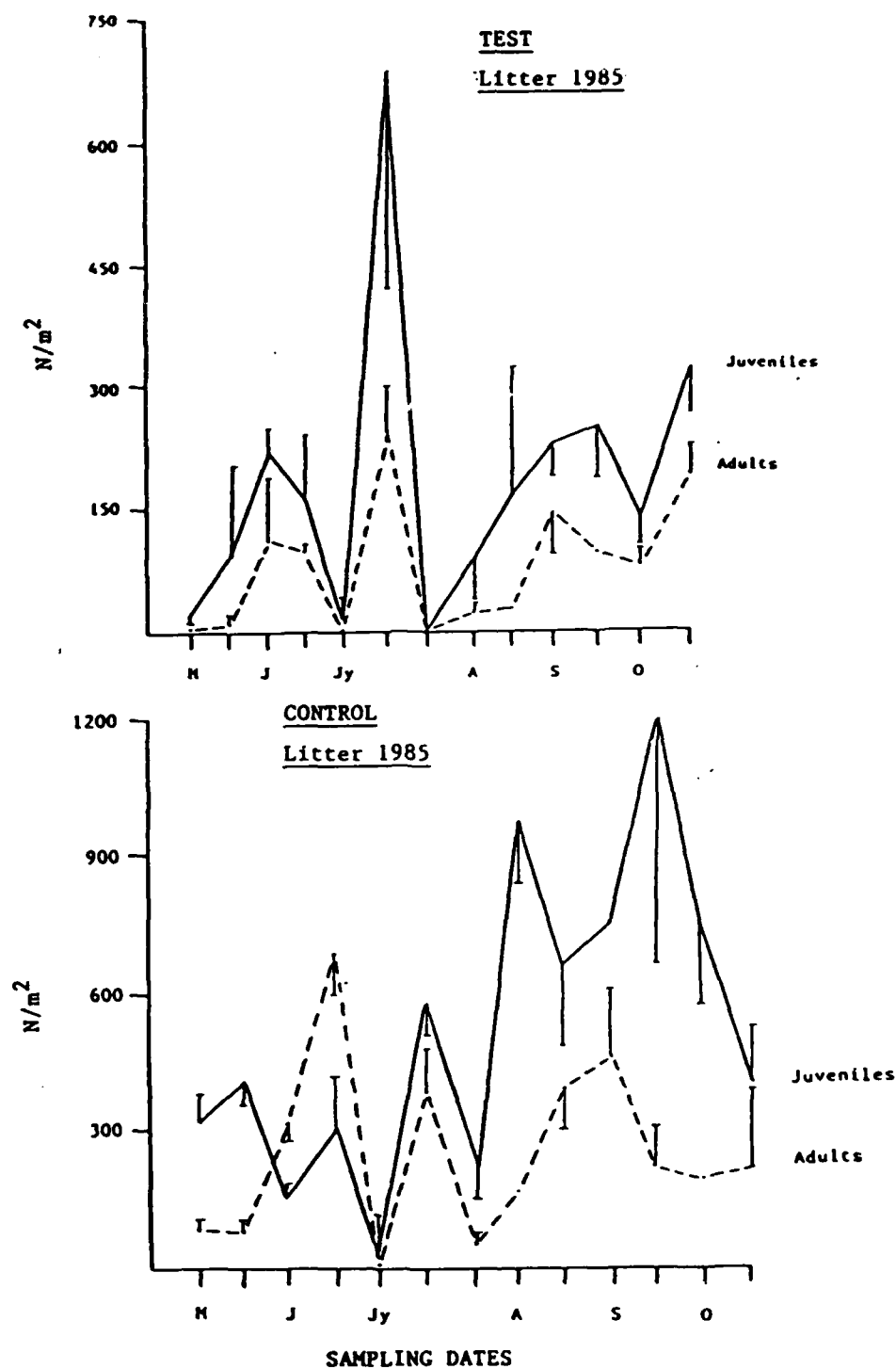


Fig. 17. Average densities \pm SE of adult and juvenile (all instars) *Isotoma notabilis* in leaf litter, Test and Control 1985. (Calandrino, thesis research).

sites showed discrepant seasonal fluctuations. Asca aphidioides (Mesostigmata) may serve as example (Fig. 18). ANOVA of differences between means, not surprisingly, showed that site as well as year effects were highly significant ($P < 0.001$).

Population structure, on the other hand, has proven constant from year to year (Figs. 19-20 illustrate the relative frequencies of developmental stages of A. aphidioides). For this species, as well as for "species A" (Mesostigmata), population structure in 1984-1985 was tested by discrete multivariate analysis (log-linear model, factors: sites, years, dates, and developmental classes). Given year and date, site and class factors were independent; i.e., the proportion of the population in a given class was not subject to site effects ($P < 0.05$ for A. aphidioides, $P < 0.005$ for species A).

Despite discrepant seasonal and year-to-year numerical fluctuation, the stage structure of these populations was thus similar between sites as well as between years, offering a valid parameter for site-comparison in future years.

Statistical treatment

i. Seasonal densities

We are in the process of expanding tests of arthropod densities by ANOVA, but statistical details will depend on successful formatting of appropriate covariate files.

The next procedural step consists of multiple regression analyses of mean abundances / date on temperature, moisture and litter mass variables. For litter-dwelling species, effects of maximum, minimum air temperatures at the time or on the day of sampling are worth investigating. In the case of I. notabilis, recently acquired data on size structure will allow

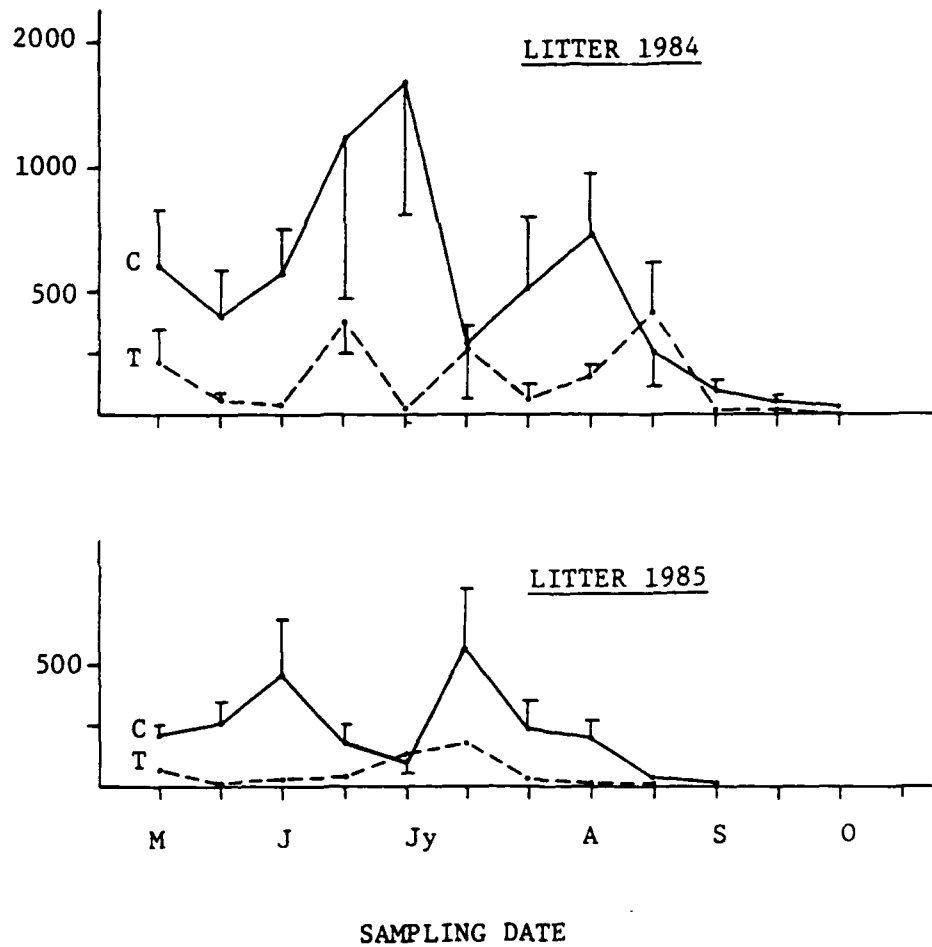


Fig. 18. Densities/ $\text{m}^2 \pm \text{SE}$ of Asca aphidioides in litter, in Test and Control (T and C) in 1984 and 1985.

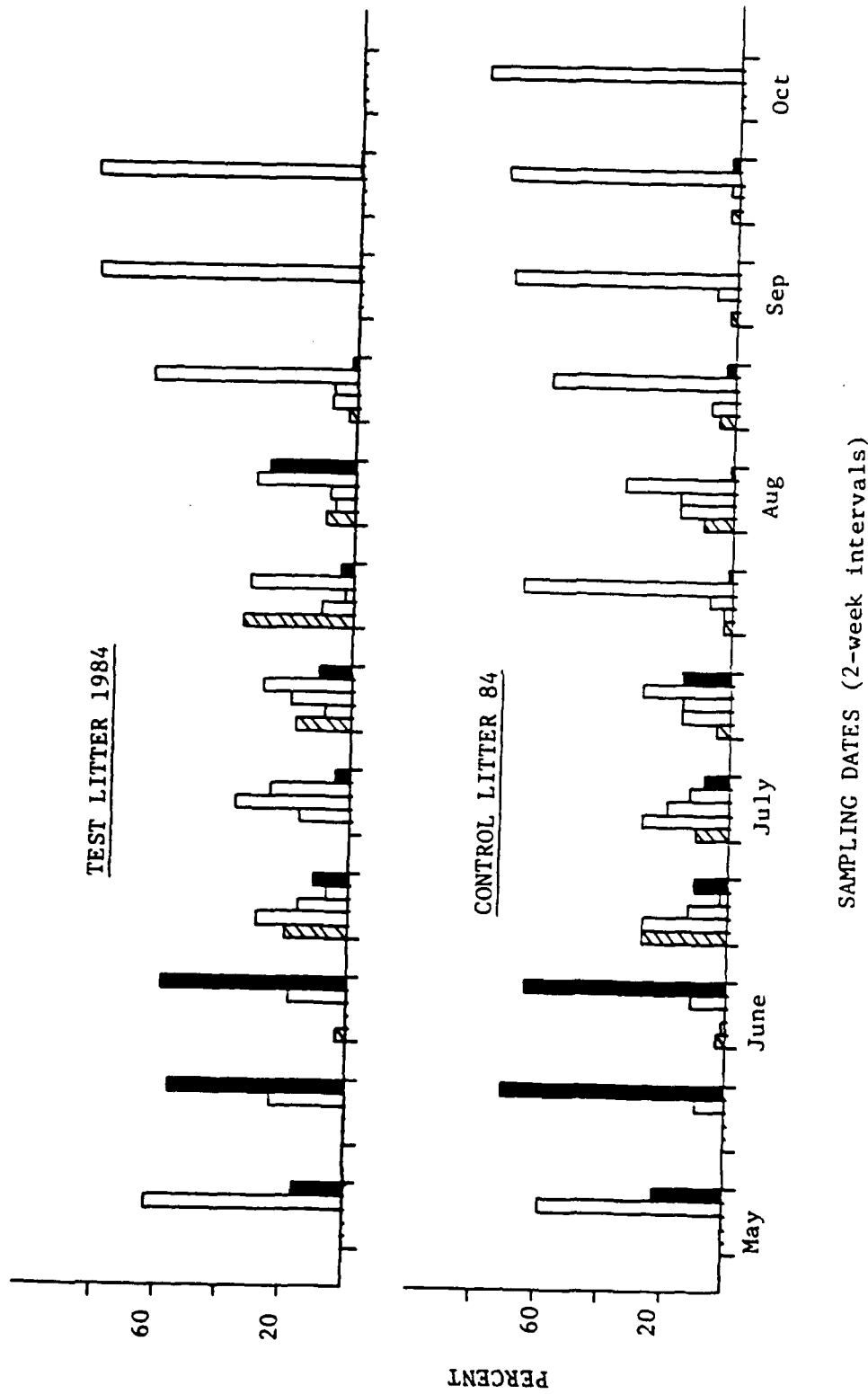


Fig. 19. Asca aphidoides: population stage structure in Test and Control, in % of total N per date. Hatched bars: larvae; black bars: gravid females; open bars from left to right: protonymphs, deutonymphs, and non-gravid females.

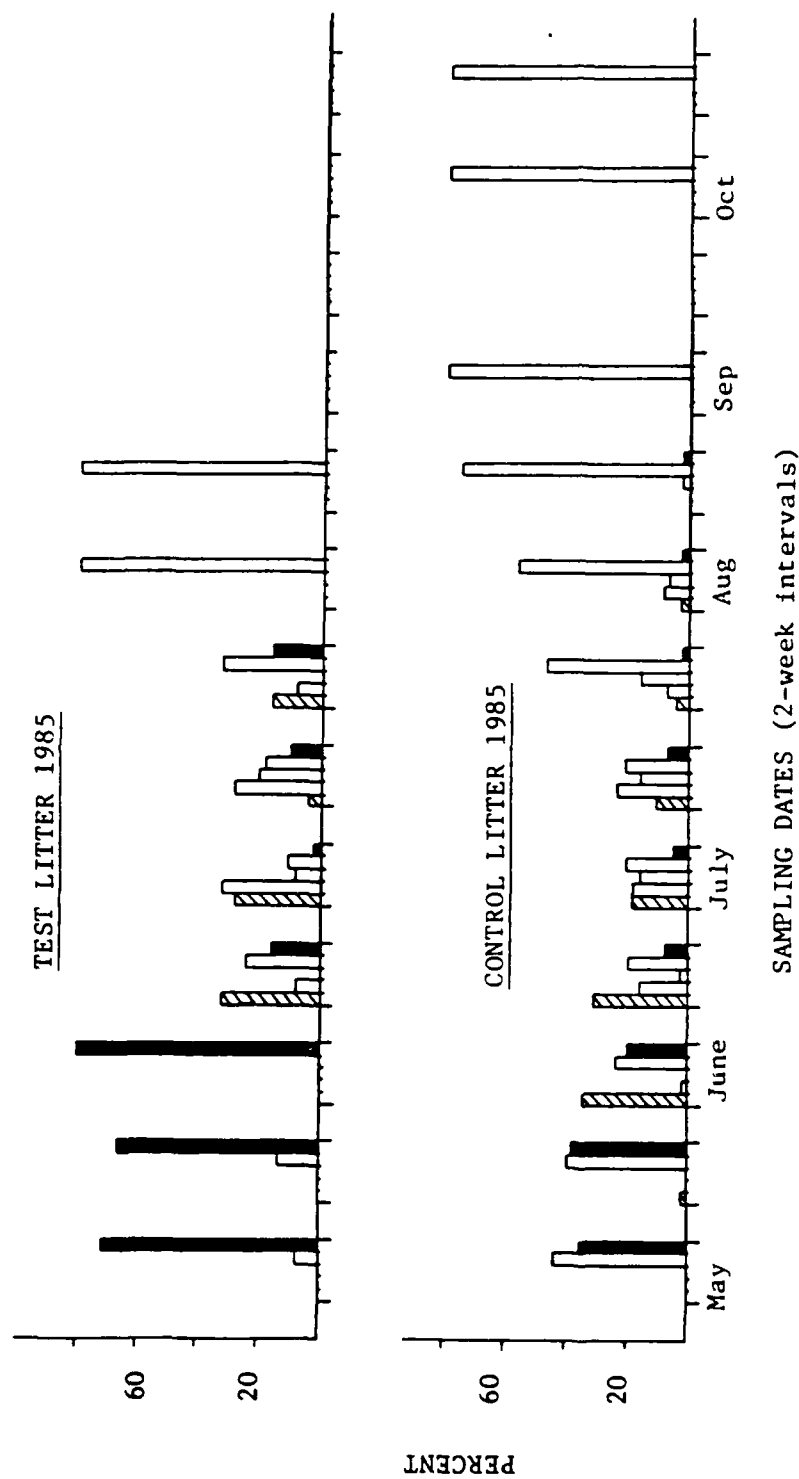


Fig. 20 . Asca aphidiods: population stage structure in 1985, in % of total N per date.

Hatched bars: larvae; black bars: gravid females; open bars from left to right: protonymphs, deutonymphs, and non-gravid females.

separate testing of life stages. Whether, and how, population structure can be used as covariate in ANOVA will be determined once the data have been fully summarized.

All of the above will aid in interpreting general relationships between edaphic or development-related factors and seasonal abundance. However, for use in any ANOVA, covariates must be sample-specific. Available for this purpose are files of:

- soil moisture: moisture samples are taken adjacent to cores used for arthropod extraction;
- litter mass: obtained directly from litter samples after extraction of arthropods;
- litter moisture: on dates when litter is not wet, estimated by regression of moisture on mass, according to data from "litter moisture samples" (water content of "arthropod litter" cannot be assessed directly).

Any variables significantly related to abundances will then be used in ANOVA of densities.

In the long-term view, we expect to use an ANOVA model which allows sensitive evaluation of changes in differences between means. It has recently been developed by J.L. Gill (in press), and is appropriate for multiple-year testing of densities over time. The main factors and interactions are as follows:

Treatments = Sites (A)
 Samples/site (ERROR 1)
 Years (B)
 Sites x Years (AB)
 Samples x Years (ERROR 2)
 Dates (C)
 Sites x Dates (AC)
 Samples x Dates (ERROR 3)
 Years x Dates (BC)
 Sites x Years x Dates (ABC)
 Samples x Years x Dates (ERROR 4).

A variety of means, differences between means and changes in differences may be tested by this model, including effects of factors conditioned on one or more others. It is thus particularly appropriate for testing potential ELF effects. We understand that a computer program (SAS) for this split-split-plot model is being developed, but anticipate some delay before it becomes available, particularly if it has to be modified to conform to our needs.

ii. Population structure

Seasonal and yearly stage frequencies will be tested by contingency tables and log-linear multivariate analysis. Counts may first be lumped to monthly totals if populations undergo any further reductions (e.g., mites), or if total numbers are low in either site. For Acari, discrete life stages form the data base. In the case of I. notabilis, delimitation of size classes, between instar I and adult, is yet to be determined.

IV. SURFACE-ACTIVE ARTHROPODA

Pit-trap data for 1987 are as yet incomplete in terms of species identification, but we can now compare two years' data obtained by means of barrier traps (1985-86). For the moment, we report on Collembola and Carabidae. Identification of 1986 mites (Abrolophus sp., Trombidium auroraense, and Nanorchestes spp.) has been completed, but these data are awaiting summary and analysis.

1. Collembola

1.1. Numbers and diversity

A summary of total numbers captured in 1985-86 may be useful for illustrating site- and year- differences. In Table 9, the total number of individuals trapped is listed for species common in either or both sites. It is apparent that sporadic appearances of normally uncommon species (e.g., P. saxatilis), and drastic year-to-year variation in activity (or density) of others, e.g., T. flavescens, make community analyses tenuous. By comparison with Table 8, it is also apparent that trap-catches are not always related to population density. The most constant species so far has been S. henshawi, the dominant sminthurid in both Test and Control.

Bray-Curtis (0.42 in 1985, 0.34 in 1986) as well as Sørensen's (0.79 in 1985, 0.77 in 1986) similarity coefficients decreased slightly from year to year. Diversity coefficients tested according to Hutcheson (1970) differed significantly between years as well as sites (Table 10).

Table 9. Total number of individuals captured of species abundant in either site, but shared between sites, 1985-86.

	TEST		CONTROL	
	1985	1986	1985	1986
SMINTHURIDAE				
<u>Sminthurinus henshawi</u>	1637	1435	2606	2934
<u>Sminthurides lepus</u>	669	236	397	375
ENTOMOBRYIDAE				
<u>Tomocerus flavescens</u>	4213	1965	842	242
<u>Orchesella hexfasciata</u>	3201	3402	1099	421
<u>Entomobrya nivalis</u>	531	1057	4	14
<u>Entomobrya comparata</u>	35	80	287	87
ISOTOMIDAE				
<u>Isotoma notabilis</u>	174	130	619	340
<u>Isotoma nigrifrons</u>	155	117	95	28
<u>Isotoma viridis</u>	250	266	18	14
HYPOGASTRURIDAE				
<u>Neanura muscorum</u>	32	88	77	59
<u>Pseudachorutes saxatilis</u>	13	0	1925	198

Table 10. Diversity indices (h) for surface-active Collembola in Test and Control, 1985-86, and level of significance of pair-wise comparisons (after Hutcheson 1970).

	h	Significance of paired tests	
		Test 86	Control 86
Test 1985	1.832	P < 0.001	-
Test 1986	1.946	-	P < 0.02
Control 1985	2.201	-	P < 0.001
Control 1986	1.902	-	-

1.2. Seasonal activity

Total numbers trapped, summarized at the family level, reflected the reduced activity and/or density of dominant species noted earlier.

Sminthurid numbers were generally reduced in Test in 1986 (Fig. 21).

Entomobryids were particularly sparse in 1986 in Control (Fig. 22), where all major contributing species, relative to 1985, occurred in lower numbers (Table 9).

We tested weekly numbers of S. henshawi, T. flavescens and O. hexfasciata captured in 1985 by ANOVA of differences between means ($N = 20$ traps/date). Site effects were significant in all cases at $P < 0.01$. Total numbers trapped, much like densities in litter or soil, were thus not prone to be useful for between-site analysis.

Regression procedures are appropriate for testing fluctuations in activity as they relate to climatic variables. So far, RH has not shown promise for explaining observed variation, probably because at some time during day or night, RH in our sites usually reaches 90-99% levels. Extremes are too few, given point-sampling at weekly intervals, to reveal any significant trends.

With respect to temperature, we have used total day and night catches $[\ln(y+1)]$ on each trapping occasion, and ratios of night/day catches, as dependent variables. Recently, total numbers were transformed to mean catches / hour, to account for seasonal changes in day and night length. Preliminary regressions showed, however, that means/hour only obscured any relationships between temperature and activity. Activity probably peaks during a brief period of time, so that total length of

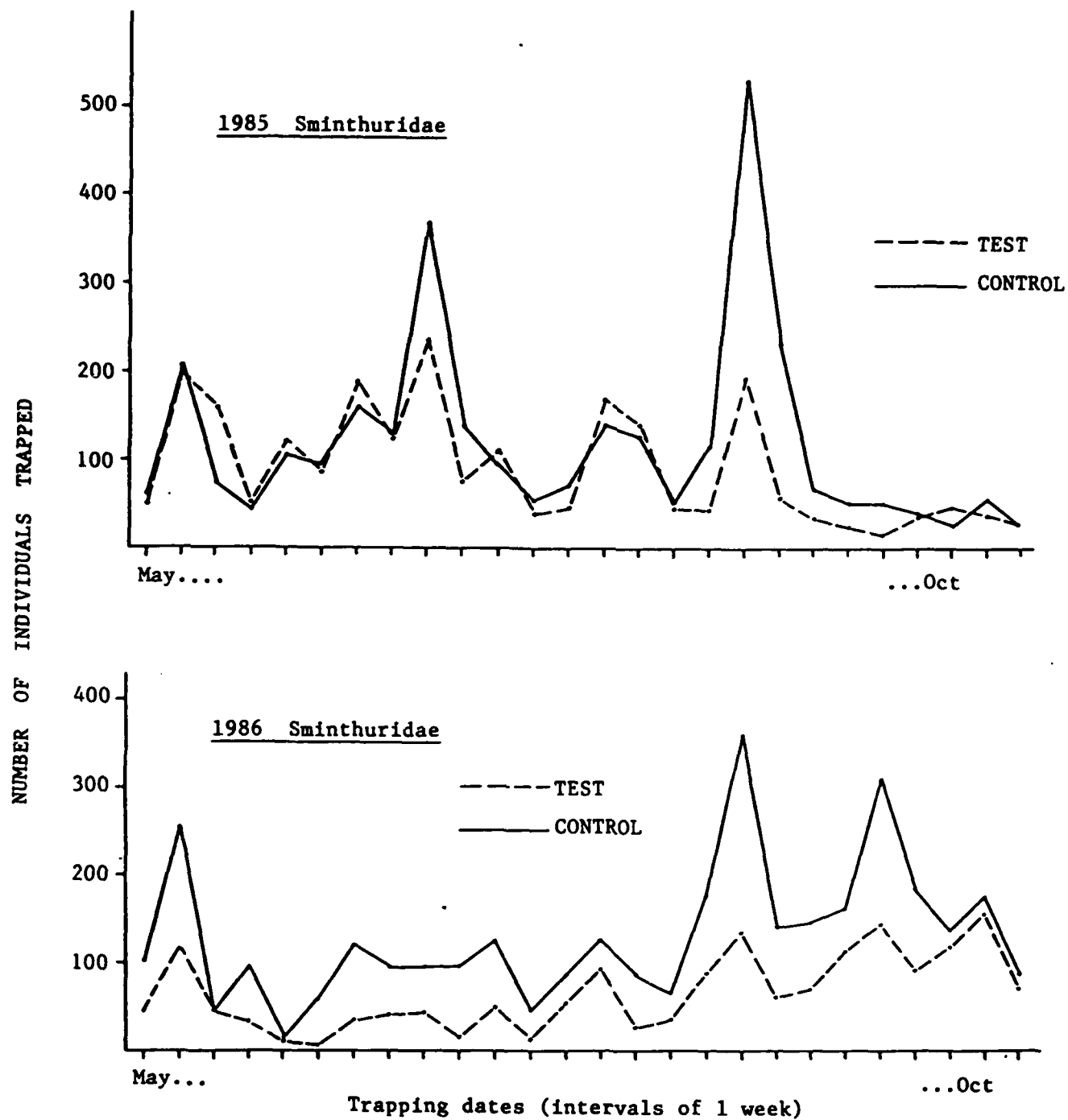


Fig. 21.. Total catches of Sminthuridae per date, 1985 and 1986.

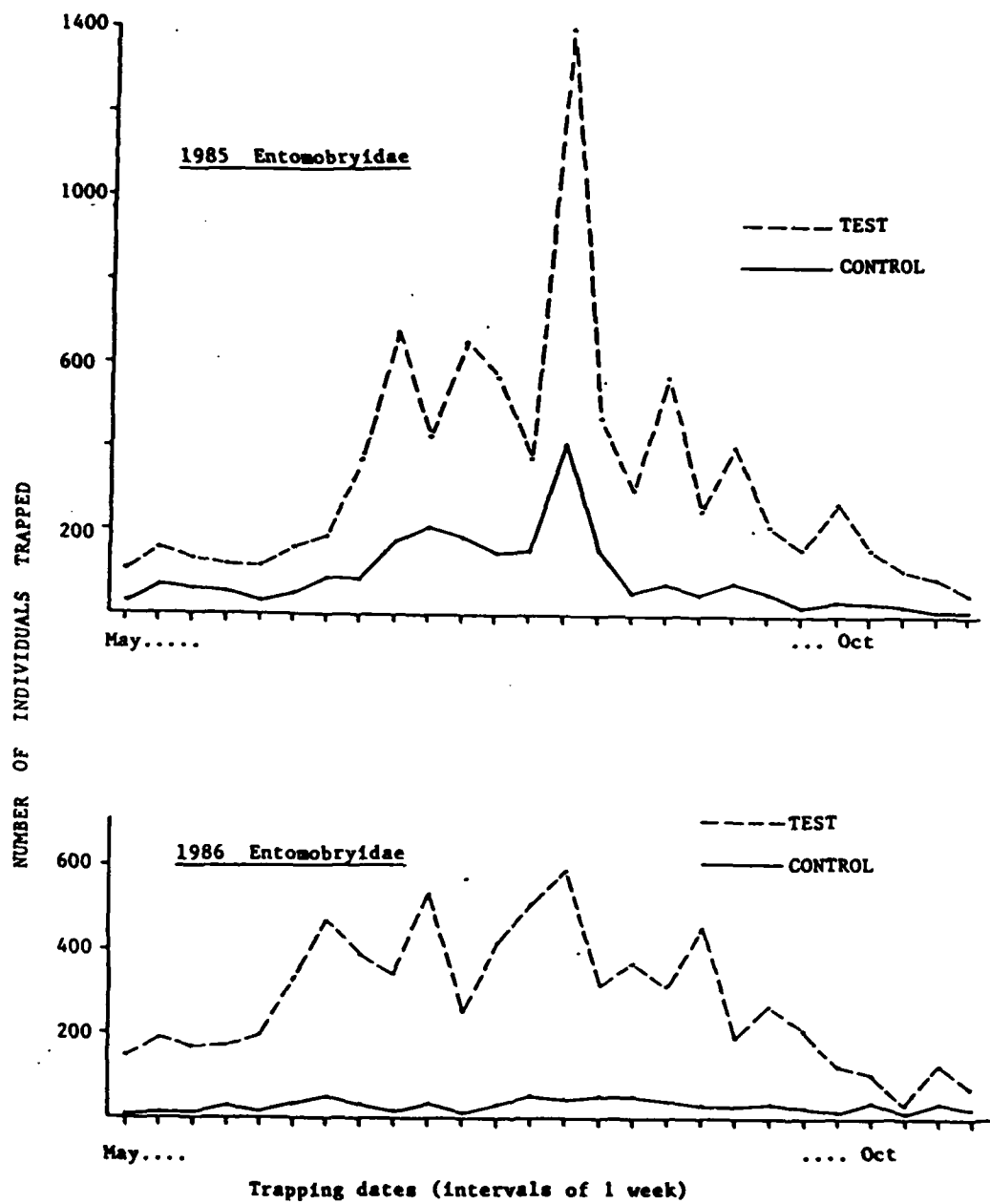


Fig. 22. Total catches of Entomobryidae per date, 1985 and 1986

day or night becomes irrelevant for interpreting numbers trapped.

Independent variables consisted of 24 arrays of temperature records, some applicable to nocturnal, some to diurnal catches, some to night/day ratios. They included, for instance, maxima, minima and means on trap days and nights, on preceding days and nights, and various temperature differences. Average temperatures were calculated from hourly Omnidata readouts between sunrise and sunset or vice versa. The same set of air temperature records were used for both sites, since Test and Control are virtually indistinguishable in this respect.

Analyses for 1986 are still ongoing, and we present results obtained from 1985 data on S. henshawi, the most frequently trapped species in both sites. Results concern only day and night catches, since none of the independent variables has yielded significant relationships with night/ day catch ratios.

Linear regressions which were significant (almost invariably in both sites) are listed in Table 11. Night catches were relatively poorly explained by any one temperature variable , trap night maxima and minima giving the best results. Maximum temperatures on nights and days immediately preceding trap nights also had significant effects on nocturnal activity fluctuations, at least statistically. Biologically, this relationship may be spurious, simply pointing to periods of warm weather in general.

For diurnal catches, several variables proved to be significant (Table 11). In particular, minima on trap days explained 44 - 56% of observed variation; i.e., as minimum temperatures increased, so did activity. Again, temperatures during nights prior to trap days were significantly related to diurnal activity.

Table 11. Linear regression parameters for day and night catches [$\ln (Y+1)$] of Sminthurinus henshawi on temperature variables (T = Test, C = Control).

Trap days immediately follow trap nights.

Regression equation			P	R ²
NIGHT CATCH				
Trap night max	T	Y= 2.1309 + 0.0844 X	0.02	0.22
	C	Y= 2.5670 + 0.0731 X	0.02	0.22
Trap night min	T	Y= 2.6496 + 0.0702 X	0.05	0.17
	C	Y= 3.0092 + 0.0618 X	0.05	0.18
Prior night max	T	Y= 1.9479 + 0.1000 X	0.02	0.23
	C	Y= 2.6392 + 0.0673 X	0.07	0.14
Prior day max	T	Y= 1.6369 + 0.0968 X	0.01	0.30
	C	Y= 2.4106 + 0.0664 X	0.04	0.20
Multiple regr.	T		0.12	0.31
	C		0.24	0.24
DAY CATCH				
Trap night max	T	Y= 2.4146 + 0.0714 X	0.008	0.29
	C	Y= 2.7834 + 0.0801 X	0.02	0.22
Trap night min	T	Y= 2.7119 + 0.0794 X	0.001	0.40
	C	Y= 3.0930 + 0.0924 X	0.005	0.32
Trap day max	T	Y= 2.2923 + 0.0615 X	0.01	0.29
	C	Y= 2.6302 + 0.0700 X	0.02	0.22
Trap day min	T	Y= 2.6254 + 0.0900 X	0.0001	0.56
	C	Y= 3.0035 + 0.1032 X	0.0006	0.44
Prior day max	T	Y= 2.4130 + 0.0561 X	0.04	0.18
	C	Y= 2.8030 + 0.0603 X	0.08	0.13
Prior day min	T	Y= 2.9291 + 0.0553 X	0.03	0.20
	C	Y= 3.3226 + 0.0651 X	0.05	0.17
Multiple regr.	T		0.012	0.54
	C		0.04	0.45

Multiple regression on the most significant temperature variables did not improve the degree of resolution in the case of night catches, and resulted in loss of significance. For day catches, trap day minima explained as much of the variation as did multiple regression on four independent variables (Table 11).

It is important, however, that in no case did the slopes of single-variable regression lines differ significantly between sites ($P < \text{or } \ll 0.05$). Responses of S. henshawi to temperature were closely similar in Test and Control, and thus represent valid behavioral parameters for site comparison.

Statistical treatment

Linear regression on temperature has so far been the best way for comparing activity of S. henshawi in Test and Control. However, we will investigate a potential relationship between trap catches and density of the species in leaf litter; the latter varies seasonally with recruitment and population growth, and could provide an important independent variable for increasing the explanatory power of regression analyses.

2. Carabidae

2.1. Dominance and diversity

Species dominance values shifted somewhat from year to year, in both sites (Table 12). Four shared species were captured in reasonably large numbers for analysis: P. melanarius, P. pensylvanicus, P. coracinus; and, particularly in 1986, S. impunctatus.

The number of species trapped remained equal in Test and Control, and total yearly catches increased in both sites (Table 12). Diversity indices, tested after Hutcheson (1970) differed significantly between years as well as sites ($P < 0.01$). Similarity indices, however, increased slightly (Bray-Curtis index from 0.42 to 0.48, Sorensen's index from 0.86 to 0.95).

Table 12. Percent dominance of surface-active Carabidae, 1985-86, and total number trapped in Test and Control.

	TEST		CONTROL	
	1985	1986	1985	1986
<u>Pterostichus melanarius</u>	50.1	46.4	7.9	8.4
<u>P. coracinus</u>	6.7	6.5	11.4	17.1
<u>P. pennsylvanicus</u>	9.5	7.1	12.1	9.4
<u>P. adstrictus</u>	0.9	0.2	10.9	6.5
<u>P. adoxus</u>	0.1	0.6	1.3	0.8
<u>P. mutus</u>	10.7	8.1	1.0	0.6
<u>Calathus ingratus</u>	1.0	0.3	5.7	0.6
<u>C. gregarius</u>	2.7	1.3	6.9	5.4
<u>Calosoma frigidum</u>	0.3	5.5	1.3	4.1
<u>Synuchus impunctatus</u>	4.8	10.4	30.4	33.9
<u>Agonum retractum</u>	0.8	0.8	0.6	0.1
<u>A. decentis</u>	0.8	0.9	3.2	2.2
<u>Harpalus fuliginosus</u>	3.5	3.5	2.4	4.4
<u>Clivina fossor</u>	2.3	1.9	0.2	0.2
<u>Cymindis cribricollis</u>	1.2	2.1	1.5	4.4
<u>Notiophilus aeneus</u>	1.4	1.4	2.0	1.0
<u>Myas cyanescens</u>	0.1	0.2	0.6	0.5
<u>Sphaeroderus lecontei</u>	0.2	0.2	0.4	0.5
<u>Agonum placidum</u>	0.1	-	-	-
<u>Trechus quadristriatus</u>	0.1	0.2	-	0.1
<u>Carabus sylvosus</u>	0.1	-	0.1	0.1
<u>Bembidion quadrimaculatum</u>	-	-	0.1	-
<u>Harpalus fulvilabris</u>	-	0.2	0.1	-
TOTAL NUMBER TRAPPED	2168	2506	2307	2639
NUMBER OF SPECIES	21	20	21	20
DIVERSITY (h)	1.858	1.947	2.270	2.149

2.2. Seasonal activity

We have documented in earlier reports that each species exhibited distinct, relatively short-term activity periods related to their reproductive cycle. Data for 1986 simply confirmed 1985 observations. For the four species of importance to this project, weekly captures are being subjected to ANOVA of differences between means in order to test the synchronicity of activity in Test and Control. The data base consists of total numbers captured per 24 hours (day + night catches on each date, N= 20 traps/date). Results will be available shortly.

2.3. Diel activity

Forest carabids in general are said to be nocturnal (Thiele 1977). We can now document that diel habits vary between years. Considering only the four abundant species shared between sites, all had a greater tendency toward diurnal activity in 1985 than in 1986 (Table 13). This is undoubtedly a result of climatic variability between years.

Table 13. Diurnality of common Carabidae in Test and Control, 1985-86.

	(DAY CATCH / TOTAL CATCH) x 100			
	TEST		CONTROL	
	1985	1986	1985	1986
<u>P. melanarius</u>	71.8	47.7	73.8	50.0
<u>P. coracinus</u>	55.5	29.4	60.5	38.7
<u>P. pensylvanicus</u>	33.5	26.8	36.0	33.6
<u>S. impunctatus</u>	50.5	37.9	36.4	31.9

Analogous to S. henshawi, catches of abundant carabids were tested for their relationship with temperature variables by linear regression. Pterostichus melanarius was selected as an example for discussion. Only data obtained during the species' main period of activity were used, so that July through September catches represent the array of dependent variables. This choice of dates to be analyzed was substantiated by dissection of females: July through September, females carry a variable number of mature eggs in their ovaries, coincident with significant surface-activity.

General conclusions concur with those arrived at for S. henshawi:

- a) ratios of night/day catches, which reflect shifts in diel habits, have so far proven intractable;
 - b) mean catches / hour, which would account for seasonally varying times of "trap exposure", did not show significant relations with temperature.
- This is not altogether surprising, since several species of carabids have been shown to be active only during a restricted number of hours of the day or night (Stubbe et al. 1984; Thiele 1977). All regression analyses were therefore based on total catches per day or night.

In Tables 14 and 15, regression parameters are listed for temperature variables significantly related to trap catches, usually in both sites, in 1985 and 1986. Several single variables yielded relatively high coefficients. Multiple regression improved R^2 only slightly in most cases, and tended to decrease the level of significance, in either site, to $P > 0.05$.

Table 14 Linear regression parameters for catches of Pterostichus melanarius [ln (Y+1)], on temperatures. (T= Test, C= Control). 1985 data.

		Regression equation	P	R ²
<u>NIGHT CATCH</u>				
Prior night max	T	Y= -2.2471 + 0.3406 X	0.02	0.44
	C	Y= -2.6416 + 0.2604 X	0.01	0.49
Prior night min	T	Y= 0.0934 + 0.2611 X	0.02	0.43
	C	Y= -0.4788 + 0.1629 X	0.05	0.32
Prior day max	T	Y= -0.8618 + 0.2043 X	0.01	0.49
	C	Y= -2.0016 + 0.1799 X	0.004	0.74
Prior day min	T	Y= 0.2562 + 0.2471 X	0.03	0.40
	C	Y= -0.4218 + 0.1586 X	0.05	0.32
Trap night max	T	Y= -0.9522 + 0.2426 X	0.03	0.37
	C	Y= -1.9193 + 0.2030 X	0.01	0.51
Multiple regr.	T		0.1	0.70
	C		0.02	0.84
<u>DAY CATCH</u>				
Trap day max	T	Y= 0.8759 + 0.1522 X	0.02	0.46
	C	Y= 0.3646 + 0.0886 X	0.2	0.17
Prior-trap day max	T	Y= 3.5208 + 0.1788 X	0.01	0.52
	C	Y= 1.8130 + 0.1582 X	0.02	0.44
Prior night - trap day max	T	Y= 2.8203 + 0.2445 X	0.002	0.63
	C	Y= 1.4004 + 0.1656 X	0.06	0.31
Multiple regr.	T		0.01	0.72
	C		0.15	0.47

Table 15: Linear regression parameters for catches of Pterostichus melanarius [$\ln(y+1)$] on air temperatures, 1986. (T= Test, C= Control).

		Regression equation	P	R ²
NIGHT CATCH				
Trap night max	T	Y= 1.0487 + 0.1385 X	0.02	0.41
	C	Y= -1.1343 + 0.1632 X	0.03	0.39
Trap night min	T	Y= 2.0383 + 0.1382 X	0.009	0.51
	C	Y= -0.1311 + 0.1781 X	0.004	0.59
Prior day max	T	Y= 1.3769 + 0.1098 X	0.06	0.30
	C	Y= -0.9076 + 0.1376 X	0.05	0.33
Trap night mean	T	Y= 1.7016 + 0.1331 X	0.01	0.49
	C	Y= -0.4504 + 0.1631 X	0.001	0.51
Prior day mean - trap night mean	T	Y= 4.2331 + 0.4379 X	0.002	0.64
	C	Y= 2.4313 + 0.4015 X	0.03	0.37
Multiple regression	T		0.053	0.70
	C		0.11	0.62
DAY CATCH				
Trap day max	T	Y= -2.1718 + 0.2705 X	0.01	0.47
	C	Y= -2.1679 + 0.2057 X	0.004	0.58
Trap day mean	T	Y= -1.7445 + 0.3079 X	0.01	0.50
	C	Y= -1.0124 + 0.1787 X	0.04	0.36
Multiple regression	T		0.04	0.51
	C		0.015	0.61

Comparison of 1985 and 1986 results (Tables 15-16) leads to the following conclusions:

a) Temperature variables significantly related to trap catches in one year may not be significant in another year. Trap night minima in 1986, for instance (Table 16), explained over 50% of observed variation in both sites, but the variable had no significant effects in 1985 (therefore not listed in Table 15).

b) Temperature differences, which quantify the relative amplitude of temperature changes, can be of importance; in general, maxima and minima on or prior to a trapping event are significant more frequently than temperature differences.

c) It is becoming clear that regression slopes within years, for any one variable, do not differ significantly between sites ($P > \text{or} \gg 0.05$). Between years, for any one site, they tend to differ due to variable temperature regimes and the carabids' response to them. Within-year site comparisons thus offer a valid tool for project purposes.

d) Single-variable regressions were generally preferable to multiple regression. However, results to date are considered preliminary for reasons given below.

Statistical treatment

Linear rather than polynomial regressions have so far given the best fit with respect to carabid activity, and will remain the primary statistical tool.

We have some evidence that males and females of some species exhibit

discrepant diel habits, and may respond differently to day and night temperatures. In P. melanarius, for instance, males have been consistently more diurnal than females in both sites, while the opposite is true of P. pensylvanicus. We have therefore divided the data base according to sex. We expect to reduce variances and increase the explanatory power of regressions by analyzing responses to temperature for males and females separately.

2.4. Fecundity

Within a given carabid species, fecundity and adult longevity can be highly discrepant when two populations from differing habitats are compared (Grüm 1984). There is little evidence, however, that populations in similar habitats vary in terms of fecundity or life cycle strategy.

We have shown for several species that periods of activity (seasonal breeding periods) are tightly synchronized in Test and Control. In an effort to find additional parameters for site comparison, we have begun to quantify the fecundity (*sensu lato*) of species of which sufficient numbers of females are captured in both sites.

As has been done by other investigators (e.g. Rivard 1964; Murdoch 1966; Barlow 1970), we may use the numbers of ripe eggs in the ovaries as an index of fecundity. Although these estimates are not always regarded as realistic (discussed by Grüm 1984), for the purposes of this project, the number of mature ova in females does provide a means of site comparison.

The effort involved in this work element is relatively small. Females are dissected, and the developmental state of the ovaries as well as the number of mature ova, if present, are recorded. Four species are of

immediate importance , although multi-year data on species common in only one site can be acquired as well.

Two years' data on P. pensylvanicus may serve as example. Given that pit-trapping allows no control over the number of females collected in a given state of gravidity, the evidence indicates that the mean number of eggs carried by Test and Control females will provide a useful parameter for analysis (Table 16). Mean number of mature ova did not differ between sites or years (t tests, $P < 0.05$), and our data generally agree with observations by Barlow (1970) on P. pensylvanicus in Canada.

Table 16. Average number of mature ova present in P. pensylvanicus females in Test and Control, 1985-86.

	<u>Mean \pm SD (N females)</u>	
	<u>TEST</u>	<u>CONTROL</u>
<u>1985</u>	12.61 \pm 4.48 (67)	11.66 \pm 4.12 (93)
<u>1986</u>	11.59 \pm 5.33 (59)	10.04 \pm 4.57 (49)

Statistical treatment

ANOVA of yearly means of mature ova will test for site, year, and site x year effects.

The number of mature eggs first increases, then decreases as the reproductive period advances. Frequency distributions can therefore be tested by contingency tables, in terms of seasonal changes in ovarian development. We anticipate lumping captured females in 2 or 4 week totals if numbers/week are low in either site .

V. LUMBRICIDAE

In 1987, earthworm samples were taken at bi-weekly intervals through July 27, at monthly intervals during the remaining season, until October 19. We were advised by IITRI that 1987 was a transitional year in terms of antenna operation, and reduced sampling frequency freed some manpower for other activities. In the bridge year of 1987 (between pre-ELF 1986 and operational year 1988), even monthly data yield sufficient information for documenting the state of populations just prior to full antenna operation.

Data from 1987 are not computerized at this time. In the following, we present summaries and analyses of major population parameters during 1984-86.

1. Community structure

Test and Control communities, with 5 and 6 species respectively, are less diverse than others in comparable forests in Europe and the British Isles (Bouche 1978; Cuendet 1984; Nordström and Rundgren 1973). In terms of species composition, similar associations of epigeics and endogeics have been documented from deciduous forests in Germany, Denmark and Sweden (Baltzer 1956; Bornebusch 1930; Nordström and Rundgren 1973).

Little is known of how diversity may change over time. In our sites, community structure varied somewhat from year to year, as a result of fluctuating densities of the component species. Diversity indices (Table 17) generally increased slightly from 1984 to 1986. Pair-wise tests of indices (after Hutcheson 1970) (Table 18) indicated that:

a) within-site differences between years were highly significant, with the exception of Test 84 / Test 85;

b) between-site differences (within years) were less, or not at all, significant. For 1985 in particular, lack of a significant difference was

partly due to increased numbers of D. octaedra in both sites;

c) using total numbers of worms/species averaged over 3 years, diversity of Test and Control communities did not differ ($P > 0.05$).

Table 17. Diversity indices for Test and Control lumbricids. Totals/species for 1984 based on 120 samples, for 1985 and 1986 on 156 samples.

N individuals / species								
Site	Year	h	A	B	C	D	E	
Test	1984	1.0102	272	497	1894	243	1	2906
	1985	1.0208	549	825	2945	288	0	4607
	1986	1.0800	592	808	2608	311	0	4319
Test average		1.0421						
Control	1984	1.0589	1234	38	1568	444	9	3293
	1985	0.9984	2885	73	1740	545	7	5250
	1986	1.1105	1666	83	1543	576	10	3878
Control average		1.0627						

Note: Test species A= D. octaedra; B= L. rubellus; C= A. tuberculata; D= A. longa; E= D. rubidus.

Control species A= D. octaedra; B= Lumbricus spp.; C= A. turgida; D= A. trapezoides; E= D. rubidus.

Table 18. Level of significance of pair-wise comparisons of diversity indices (see Table 17) for Test and Control lumbricids.

	Test 85	Test 86	Cont 84	Cont 85	Cont 86
Test 84	NS	< .001	< .01	-	-
Test 85	-	< .001	-	NS	-
Test 86	-	-	-	-	< .05
Cont 84	-	-	-	< .001	< .001
Cont 85	-	-	-	-	< .001
Test average / Control average , $P > .05$					

In the long-term view, comparison of diversity indices seems advisable, if only to quantify within-site yearly differences and their relative magnitude with respect to between-site changes. Pre- and post-ELF averages may be the most appropriate data base for these long-lived, slow-developing species. Should major deviations occur, other analyses can be used to pinpoint their probable cause by quantifying single species' numerical fluctuations.

2. Horizontal distribution

2.1. Distribution over quadrats

In 1983, during the beginning months of the project, all 20 quadrats/site were sampled on four occasions. In 1984, we began to sample only 10 quadrats (arbitrarily choosing all even-numbered quadrats in each site). Either group of quadrats is, in fact, satisfactory for monitoring lumbricid populations. Within 95% confidence limits, mean numbers of any one species did not differ between odd and even quadrats (Table 19). In 1987, in order to re-distribute the pressure of physical site disturbance, we therefore switched to sampling of odd-numbered quadrats.

Table 19. Mean number of individuals of each species (\pm 95% CL) obtained from odd- and even-numbered quadrats in 1983 (N = 40).

TEST:	D. octaedra		L. rubellus		A. tuberculata		A. longa	
	even	odd	even	odd	even	odd	even	odd
mean	10.1	8.3	16.2	18.4	57.0	59.1	7.5	7.0
(\pm 95% CL)	(3.8)	(4.2)	(3.9)	(4.2)	(11.1)	(9.9)	(1.6)	(2.2)
CONTROL:	D. octaedra		Lumbricus spp.		A. turgida		A. trapezoides	
	even	odd	even	odd	even	odd	even	odd
mean	54.9	56.7	1.1	0.6	86.3	101.0	15.8	15.7
(\pm 95% CL)	(10.7)	(9.2)	(0.9)	(0.6)	(17.9)	(12.9)	(3.9)	(3.7)

The distribution of species was further analysed by multivariate ANOVA of worm counts (all depths summed) obtained for 1984, 85 and 86. For Control, Lumbricus spp. were lumped as usual; the rare D. rubidus was excluded from both sites' data. For each site, arrays of four species/ date/ quadrat thus formed the data base. Omitting much detail, Table 20 lists significance levels for main factors and their interactions.

Lumbricus rubellus in Test and [L. rubellus + L. terrestris] in Control were the exceptions with respect to quadrat effects; for all others, the effect was highly significant (Table 20). However, mean number of worms of any one species could not be correlated with the location of quadrats within a site; i.e., no gradients within site confines became apparent. Neither peripheral nor centrally located quadrats were distinguishable by extremes in terms of earthworm populations.

Table 20. F values and levels of significance for main factors and interactions in multivariate ANOVA of earthworm distribution over Test and Control sites.

TEST:		D. octaedra		L. rubellus		A. tuberculata		A. longa	
Source	df	F	α	F	α	F	α	F	α
Years	2	3.04	.05	5.13	.007	5.13	.007	0.71	.49
Dates	11	1.94	.04	1.31	.22	1.80	.05	2.24	.01
Quadrats	9	5.16	.0001	1.74	.08	5.08	.0001	5.14	.0001
Y x Q	18	1.07	.39	1.21	.25	1.34	.17	0.47	.97
D x Q	99	0.86	.79	1.11	.27	1.04	.39	0.98	.55
Y x D	22	1.11	.34	1.98	.008	1.14	.30	2.47	.0005
Error	199								

CONTROL:		D. octaedra		Lumbricus sp.		A. turgida		A. trapezoides	
Source	df	F	α	F	α	F	α	F	α
Years	2	34.46	.0001	3.37	.037	6.36	.002	7.44	.0008
Dates	11	6.72	.0001	1.95	.036	2.02	.028	2.95	.001
Quadrats	9	9.90	.0001	0.82	.6	11.39	.0001	5.25	.0001
Y x Q	18	2.51	.001	0.93	.5	2.35	.002	1.47	.1
D x Q	99	1.01	.5	0.80	.9	0.63	.99	1.42	.02
Y x D	22	2.97	.0001	0.63	.9	2.26	.002	1.33	.16
Error	199								

Year effects, as may be expected in view of diversity shifts discussed earlier, were significant for all species except A. longa. Results for D. octaedra in Control generally showed the highest levels of significance, including Year x Quadrat and Year x Date interactions. This abundant epigeic also exhibited great density variations within and between years (section 4).

Partial correlation coefficients derived from this ANOVA indicated that the distributions of none of the possible species pairs was correlated to any significant extent (highest coefficient of 0.37, for D. octaedra and A. tuberculata in Test). All species, however, and in all years, were highly aggregated, indices of dispersion (Southwood 1978, p.39) lying well outside the limits of Chi square.

It is unlikely that either degree of aggregation or overall distribution of species will undergo detectable changes in the future. In summary, the distribution of a given species within each site was essentially independent of that of others. Although significant quadrat effects indicated uneven dispersion of earthworms over the sites, no gradient seemed to exist. Sampling of either even or odd quadrats, neither being clustered in any particular way, should yield a valid, site-specific estimate of population parameters.

2.2 Horizontal distribution of epigeics

Conceptually, distribution of the epigeic D. octaedra should be correlated to the distribution (relative mass or thickness) of leaf litter. However, we have not been able to prove this relationship statistically. Multiple regression of number of worms in litter on litter mass and moisture has not yielded significant results. When litter is dry, D. octaedra retreats to the A horizon irrespective of litter cover. During rainy periods, factors other

than, or in addition to, litter mass seem to govern the distribution of the species.

In 1988, we intend to make one more attempt to quantify this potential relationship. To date, we have estimated "worm litter" mass by averaging the masses of "litter moisture" and "arthropod litter" samples. The three $1/16 \text{ m}^2$ samples are taken contiguously from an area of approximately equal litter cover. A check of this estimation method was performed with 20 samples, by linear regression of worm litter mass on mean mass of [arthropod + moisture samples] ($P < 0.01$, $r = 0.85$). However, in 1988 we will save litter samples after earthworm extraction, and thus obtain more precise dry mass values as independent variables for analysing the horizontal distribution of D. octaedra and L. rubellus.

3. Vertical distribution

It is well known that different species of lumbricids prefer certain strata of the forest floor, and that the vertical distribution of burrowing species varies more widely with season than that of surface-dwellers (Wilcke 1953; Rundgren 1975; Satchell 1980). We have quantified vertical movement of major Test and Control species with respect to moisture variables.

a) Dendrobaena octaedra inhabits leaf litter whenever moisture is adequate, and retreats to the A horizon during dry periods. In order to quantify this behavior in Test and Control, proportion of the total population present in litter was regressed on percent litter moisture. Dependent variables were transformed to $\log [p/(1-p)]$ (p = proportion). Regressions were significant for both sites ($P < 0.01$, $r_{\text{Test}} = 0.71$, $r_{\text{Control}} = 0.71$), and yielded the following parameters:

$$Y_{\text{Test}} = -1.0338 + 0.0103X,$$

$$Y_{\text{Control}} = -1.1397 + 0.0108X.$$

Regression slopes did not differ between sites ($P > 0.3$). Vertical stratification of D. octaedra in response to moisture thus furnishes a good behavioral indicator for between-site analysis.

b) Lumbricus rubellus in Test is a less constant litter inhabitant than D. octaedra, since generally less than 40% of the population are present in litter at any time. However, it too invades litter if it is moist: linear regression was significant at $P < 0.01$, with $r = 0.70$ and $Y = -1.8824 + 0.0090X$.

c) Aporrectodea spp.: for the three Test and Control endogeics, we have qualitatively documented that A. trapezoides behaves differently from the other two species. It tends to retreat readily and deeply, to the point of being absent from the A horizon entirely (Fig. 23). Aporrectodea turgida and A. tuberculata tend to remain in the A and upper B horizons, their depth distribution being remarkably similar over the seasons (Figs. 24 and 25). Summarized by year, these differences are evident in the mean proportion of each population dwelling in the A horizon: while the summer drought of 1985 resulted in insignificant average declines, rainfall deficits of 1986 elicited much more pronounced vertical migration in A. trapezoides than in either of the other species (Table 21).

Table 21. Average proportion of endogeic earthworm populations present in the A horizon in Test and Control (means \pm SE; N = 12 dates in 1984, 13 dates in 1985 and 1986).

	1984	1985	1986
<u>A. tuberculata</u> (T)	0.790 \pm 0.031	0.746 \pm 0.050	0.667 \pm 0.055
<u>A. turgida</u> (C)	0.806 \pm 0.038	0.782 \pm 0.052	0.760 \pm 0.047
<u>A. trapezoides</u> (C)	0.635 \pm 0.063	0.649 \pm 0.082	0.469 \pm 0.081

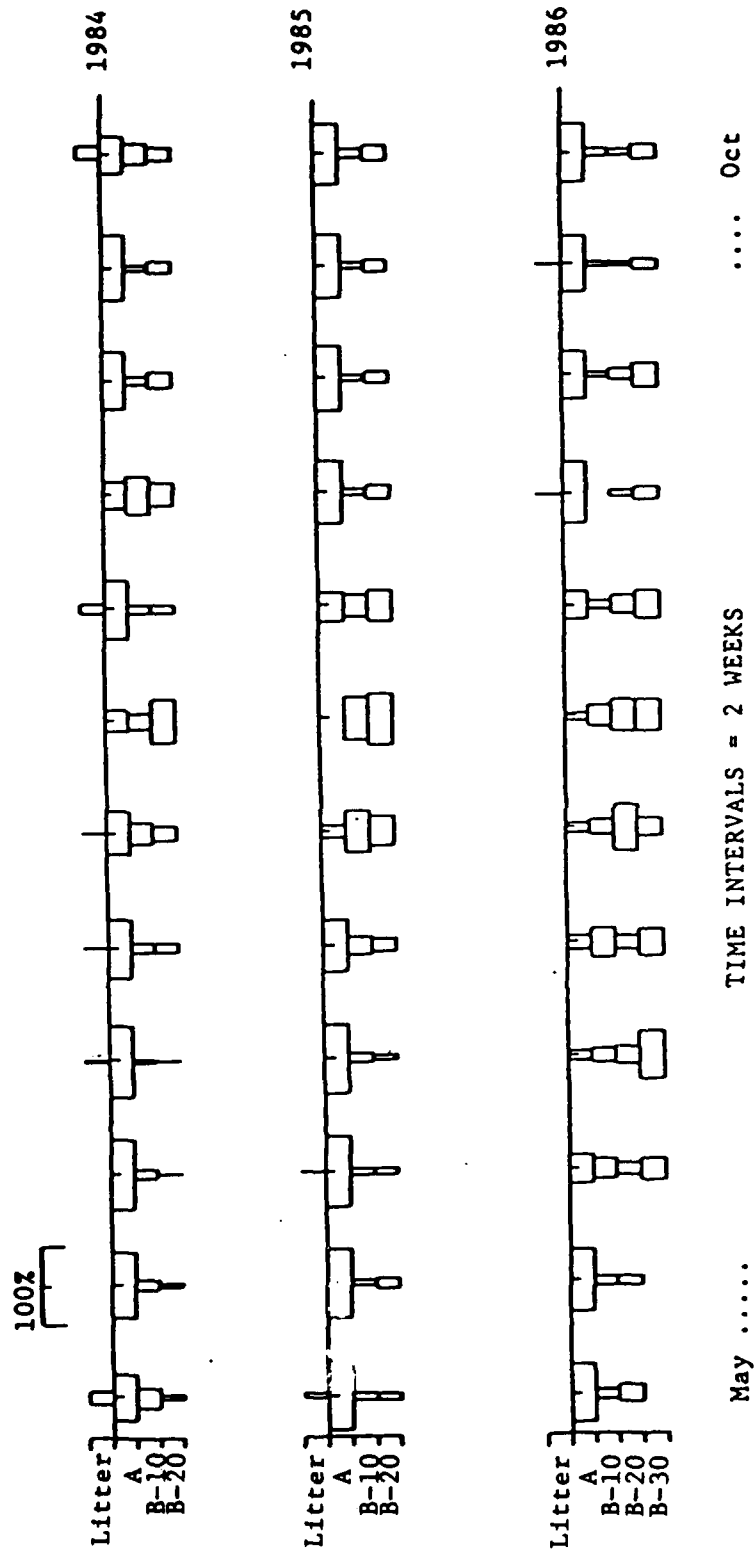


Fig. 23. Depth distribution (in % of total worms) of A. trapezoides in Control, 1984-86

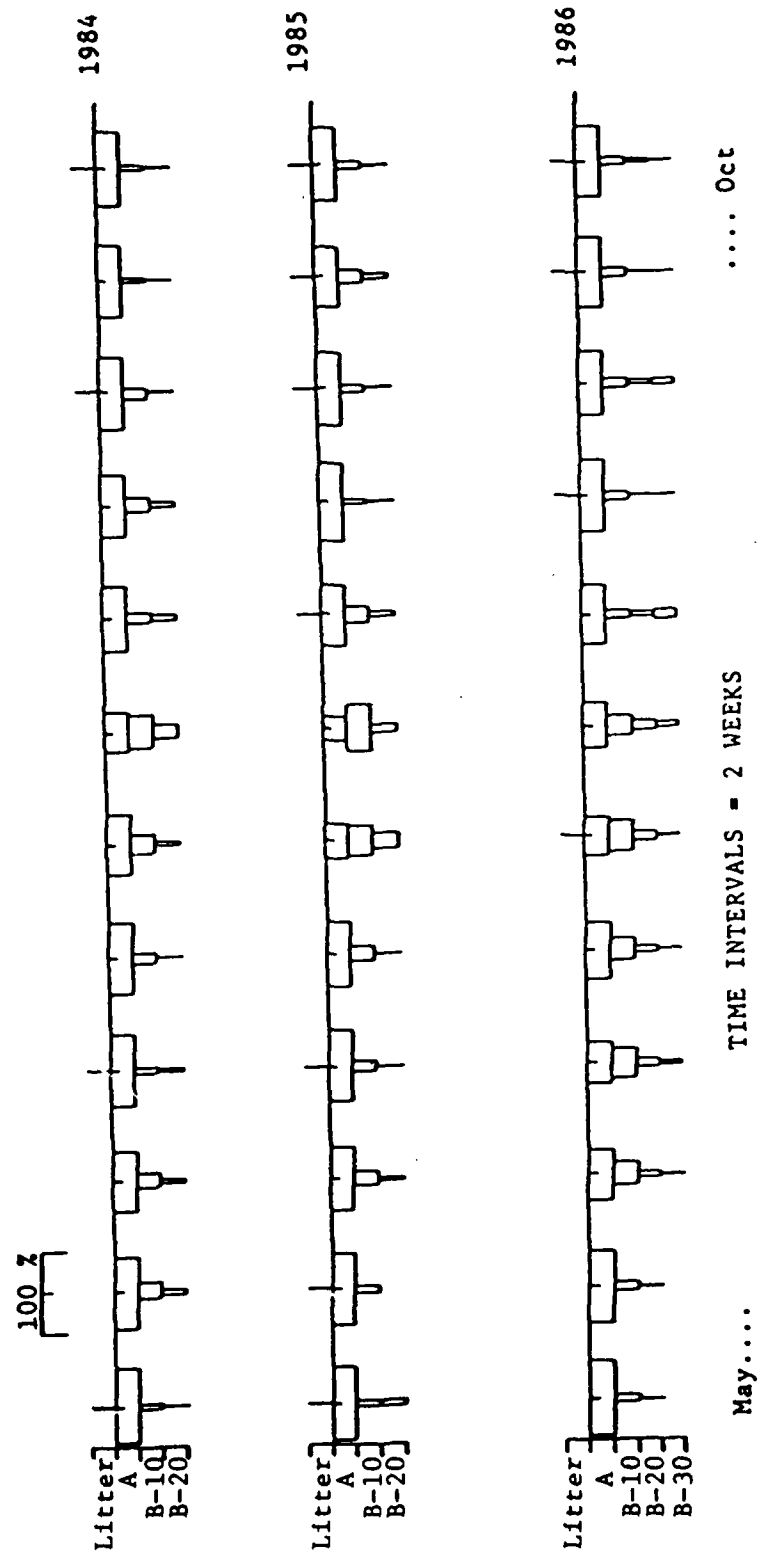


Fig. 24. Depth distribution (in % of total) of *A. turgida* in Control, 1984-86.

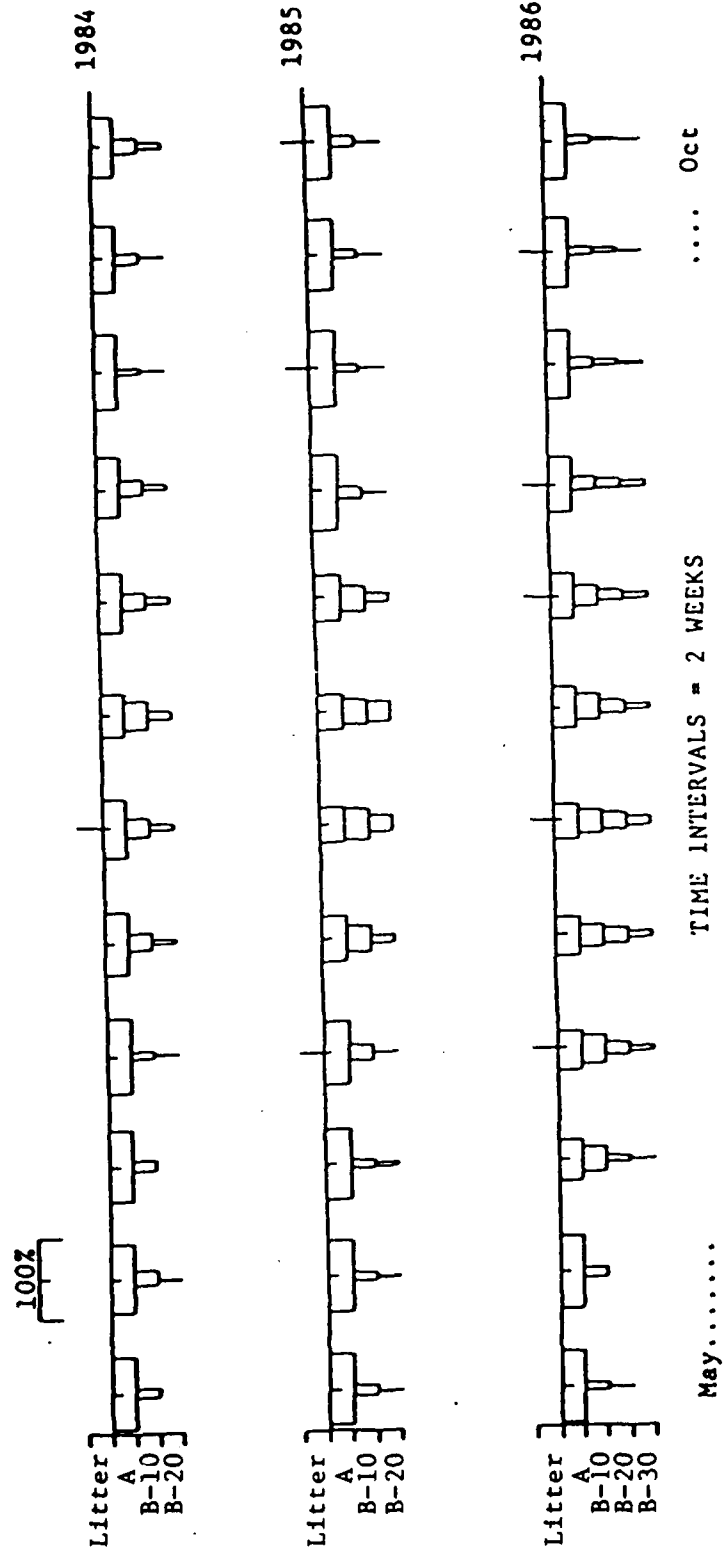


Fig. 25. Depth distribution of A. tuberculata (in % of total/ date) in Test, 1984-86.

The proportion of each population recovered from the A horizon on each date can be used as indicator of potential moisture stress, irrespective of their relative distribution at lower depths. Regression of $\log [p/(1-p)]$ on average A horizon moisture (Fig. 26) confirmed the trends discussed above. Regression slopes for A. turgida and A. tuberculata did not differ significantly ($P > 0.3$); in both species, approximately 50% of the population ($\log Y = 0$) has retreated to the B horizon when soil moistures fall to 15 - 18%. Aporrectodea trapezoides is more sensitive to dryness, half of the population being found in the B horizon when A horizon moisture declines to approximately 25%.

Regression coefficients are not particularly high for any of these endogeics (see Fig. 26). In part, variation is introduced by long-term residual effects of moisture stress. Some individuals of A. trapezoides, for instance, remain in estivation in the lower B horizon long after re-wetting of the topsoil. There is also evidence that responses to moisture and/or temperature vary with earthworm size or developmental stage (our data and Nordström

1975), in endogeics as well as litter-dwellers. Small immatures of endogeics tend to remain near the surface even during drought; on the other hand, adult D. octaedra tend to remain in the A horizon even when litter is wet.

Statistical treatment

Preliminary regressions have shown that sample-specific data of depth distribution vs. moisture and depth of the A horizon are too variable to yield significant results. Rather, we will continue to use mean vertical distribution per date and species as dependent variables in multiple regression. In addition to proportional distribution of the total population, adults and immatures will be tested separately. We hope to improve regression coefficients by making this distinction.

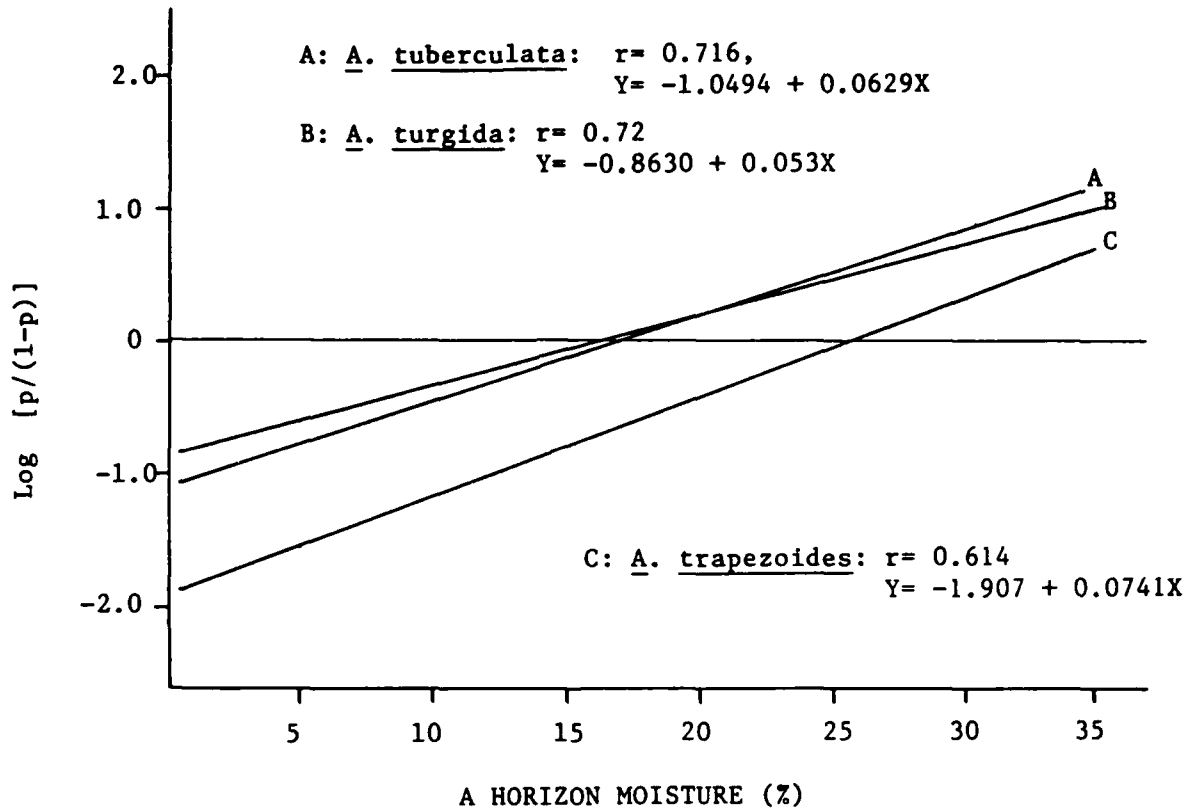


Fig. 26 . Regression lines for proportion of worms in the A horizon (mean proportion per date) on mean A horizon moisture per date, for three endogeic Aporrectodea spp. (N= 38 per species).

Independent variables will include A horizon temperature (available for 1985 and 1986) in addition to average moisture. Temperature files consist of means for the weeks prior to each sampling day, and of average temperatures on the day of sampling. For D. octaedra and L. rubellus, we expect that air temperature, particularly the maxima prior to and on the day of sampling, will yield the more significant results.

4. Density and biomass

We routinely obtain density and mass estimates for all species in order to quantify changes in community structure. Four species are of particular interest to project goals, either for pre- and post-ELF analysis within Test, or for between-site comparison. Average yearly mass and abundance data for these species are summarized in Table 22.

Table 22. Mean yearly biomass (g, preserved wet mass) and density (N) of abundant Test and Control species; N = 120 for 1984; N = 156 for 1985-86.

		M e a n s \pm 95% CL		
		1984	1985	1986
<u>D. octaedra</u> (T)	g:	1.60 \pm 0.42	1.62 \pm 0.40	2.06 \pm 0.47
	N:	38.56 \pm 10.97	56.32 \pm 13.66	62.24 \pm 12.12
<u>D. octaedra</u> (C)	g:	6.85 \pm 1.04	9.04 \pm 1.09	9.36 \pm 1.11
	N:	180.40 \pm 26.58	302.26 \pm 39.95	171.28 \pm 18.78
<u>L. rubellus</u> (T)	g:	12.13 \pm 1.96	13.28 \pm 1.82	12.96 \pm 1.70
	N:	86.67 \pm 14.23	84.72 \pm 9.07	85.02 \pm 8.99
<u>A. tuberculata</u> (T)	g:	83.23 \pm 7.61	79.20 \pm 8.05	69.22 \pm 6.09
	N:	277.33 \pm 23.89	302.56 \pm 27.57	266.77 \pm 18.81
<u>A. turgida</u> (C)	g:	42.48 \pm 4.43	29.73 \pm 3.76	25.98 \pm 3.10
	N:	220.67 \pm 32.28	202.16 \pm 24.13	158.86 \pm 18.58

Lumbricus rubellus showed the greatest population stability, density and mass remaining essentially unchanged over three years.

Aporrectodea turgida and A. tuberculata experienced population declines from 1984 to 1986, significantly so in A. turgida ($P < 0.01$). Mean biomass of D. octaedra increased in both sites, but a consistent numerical increase was recorded only for Test. The very abundant and variable Control population reached a significant peak in 1985, then returned to, approximately, the density level of 1984 (Table 22).

Estimates of changes detectable at the 5% level vary with species, ranging from approximately 15 to 28% increases or decreases over 1986, in mass or numbers, given present replication. These parameters are thus not particularly sensitive, yet yearly means are undoubtedly useful as indicators of population trends over the long term.

With respect to seasonal abundance, D. octaedra is the main candidate for analysis. In both sites, populations are highly variable, yet show similar increases and declines (Fig. 27). We tested differences between mean densities (using $\log(y+1)$) and mean biomass/ date by ANOVA, and found that site effects were highly significant ($P < 0.0001$) in both cases. As expected, so were effects of years and dates. We must conclude that seasonal density and mass per se are too variable to furnish indicators of potentially subtle perturbation.

The specimen base from which abundance and mass estimates are derived yields much more than overall numbers. We do not yet know whether other population characteristics can be of significant use as covariates for ANOVA of seasonal fluctuations. In principle, patterns of reproduction and emergence, modified seasonally by climatic factors, should provide these covariates. Formatting of the appropriate files, however, has proven difficult because of the variable time lag between "cause and effect". For the moment, we leave the matter pending.

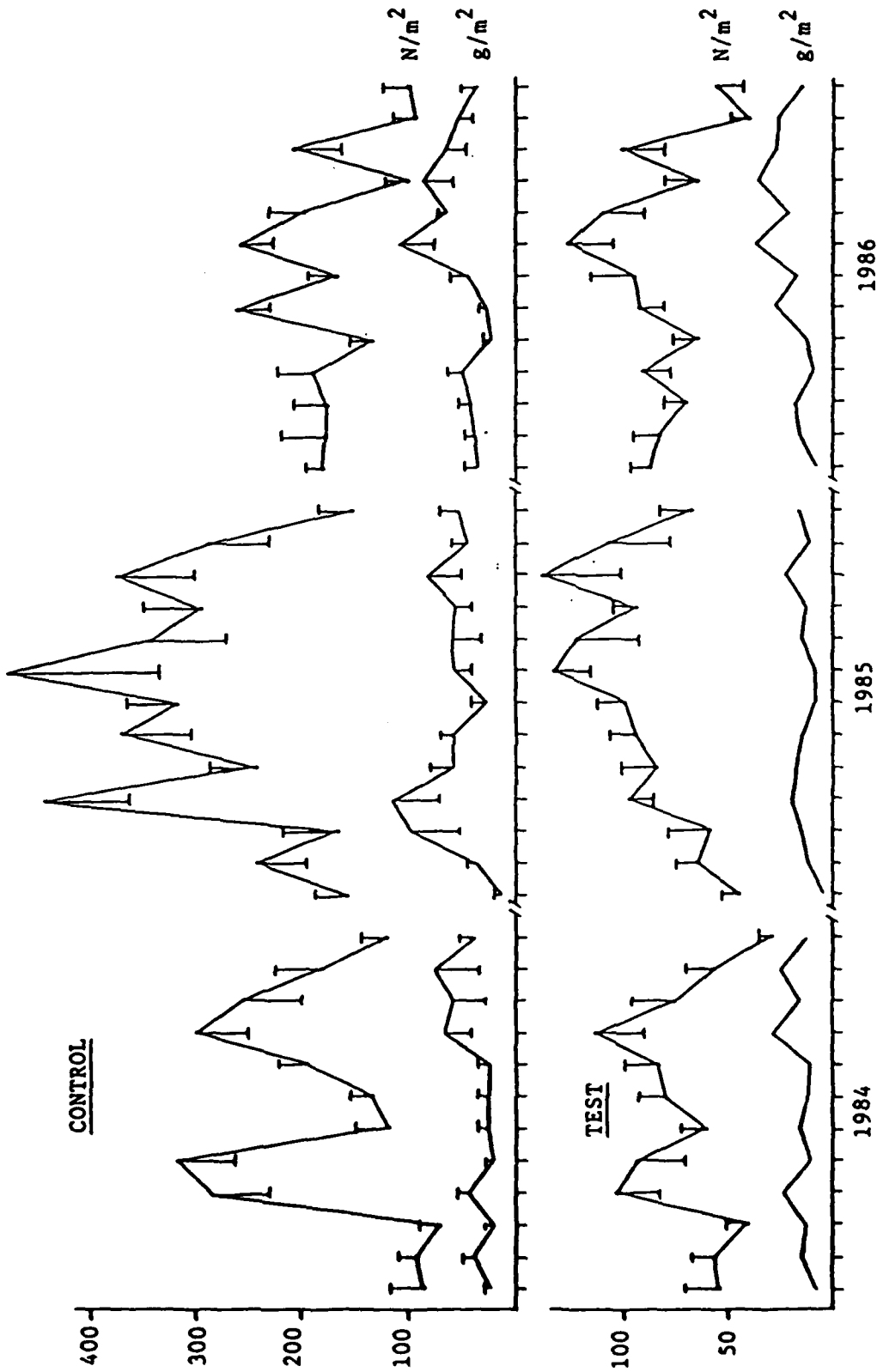


Fig. 27. Density and biomass of *D. octaedra* in Test and Control over 3 years (sampling intervals are 2 weeks apart, from May to October).

5. Reproduction

The distinctive rainfall patterns of 1984-86 affected reproductive activity in species-specific ways. Yearly average individuals in the clitellate state, based on percent of all adults collected per date, are given in Table 23.

Table 23. Average percent of adults in the clitellate state (N = 12 dates in 1984, N = 13 dates in 1985-86.

	Means \pm 95% CL		
	1984	1985	1986
<u>D. octaedra</u> (C)	64.4 \pm 21.4	21.7 \pm 7.2	45.4 \pm 17.0
<u>D. octaedra</u> (T)	50.0 \pm 22.9	19.5 \pm 13.9	25.0 \pm 12.1
<u>L. rubellus</u> (T)	85.5 \pm 9.0	69.1 \pm 15.9	69.1 \pm 10.5
<u>A. tuberculata</u> (T)	48.7 \pm 13.8	17.6 \pm 6.7	10.3 \pm 4.5
<u>A. turgida</u> (C)	55.6 \pm 6.9	45.5 \pm 6.5	31.1 \pm 11.1
<u>A. trapezoides</u> (C)	45.5 \pm 15.1	32.5 \pm 16.3	19.8 \pm 9.0

Clearly, 1984 was the most propitious year for all species. Lumbricus rubellus was the most resistant to variable precipitation, approximately 70% of adults being sexually mature in 1985 and 1986. The most severe effects of the 1986 drought were apparent in large-bodied endogeics, A. tuberculata and A. trapezoides.

Seasonal fluctuations of percent adults in reproductive condition are illustrated in Figs. 28-30. Despite considerable variation, certain patterns emerge:

Dendrobaena octaedra tended to be non-reproductive in early spring. In 1984 (Fig. 28) at least 75% of adults had become clitellate by early July; a brief depression in soil moisture had little effect on them, and virtually all adults remained reproductive through the end of the season in both sites. In 1985, a June increase in the proportion of clitellates was curtailed by summer drought,

and recovery did not occur in the remainder of the season (Fig. 29). In 1986, the species again did not develop its full reproductive potential, appearance of clitellates being delayed until late June. Greater moisture retention of Control soils now seemed to contribute to differences between sites: July through October of 1986, clitellate adults were less frequent in Test than in Control (Fig. 30).

Of the three endogeics, A. turgida was the most resistant as well as the fastest to respond to changing moisture. Similar to A. turgida, 50 to 75% of A. tuberculata may be reproductive under suitable conditions (Fig. 28 , 1984). The latter, however, recovered more slowly and to a lesser extent than A. turgida (Figs. 28-30).

In all years, A. trapezoides exhibited more drastic responses to decreasing soil moisture than other endogeics. Prolonged drought resulted in absence of clitellates in 1985 and 1986, and even brief moisture declines were followed by reductions in the proportion of reproductive adults (Fig. 28 , 1984).

Lumbricus rubellus proved to be the most impervious of all species. Although reductions in clitellate adults occurred at various times, between 50 and 100% were clitellate on most dates. The short-term variability evident in Figs. 28-30 indicates that the species is able to respond quickly to moisture changes. Resistance to dryness may be a major factor in the long-term population stability of L. rubellus (Table 22 , section 4).

Relationships between moisture and reproductive state of adults, despite obvious trends, are not entirely straightforward. Accounting for the time delays between moisture reduction and disappearance of clitellates (in endogeics, for instance) requires data manipulations which can become dangerously arbitrary. In addition, intrinsic tendencies (e.g., low proportion of sexually mature individuals of D. octaedra in spring) are independent of soil moisture

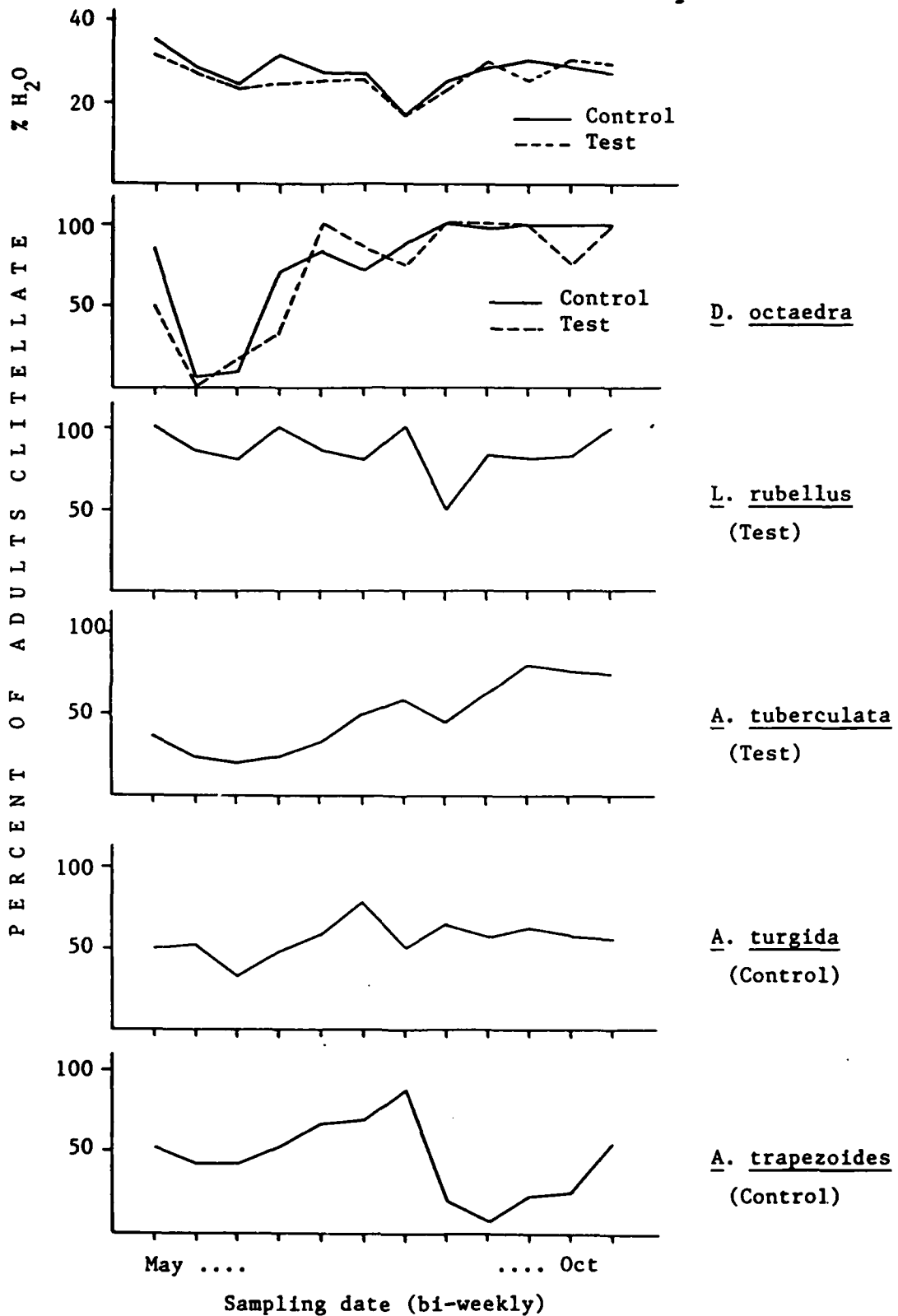


Fig. 28 . Percent moisture of A horizon (uppermost graph) and percent of adults in the clitellate state , for Test and Control lumbricids in 1984.

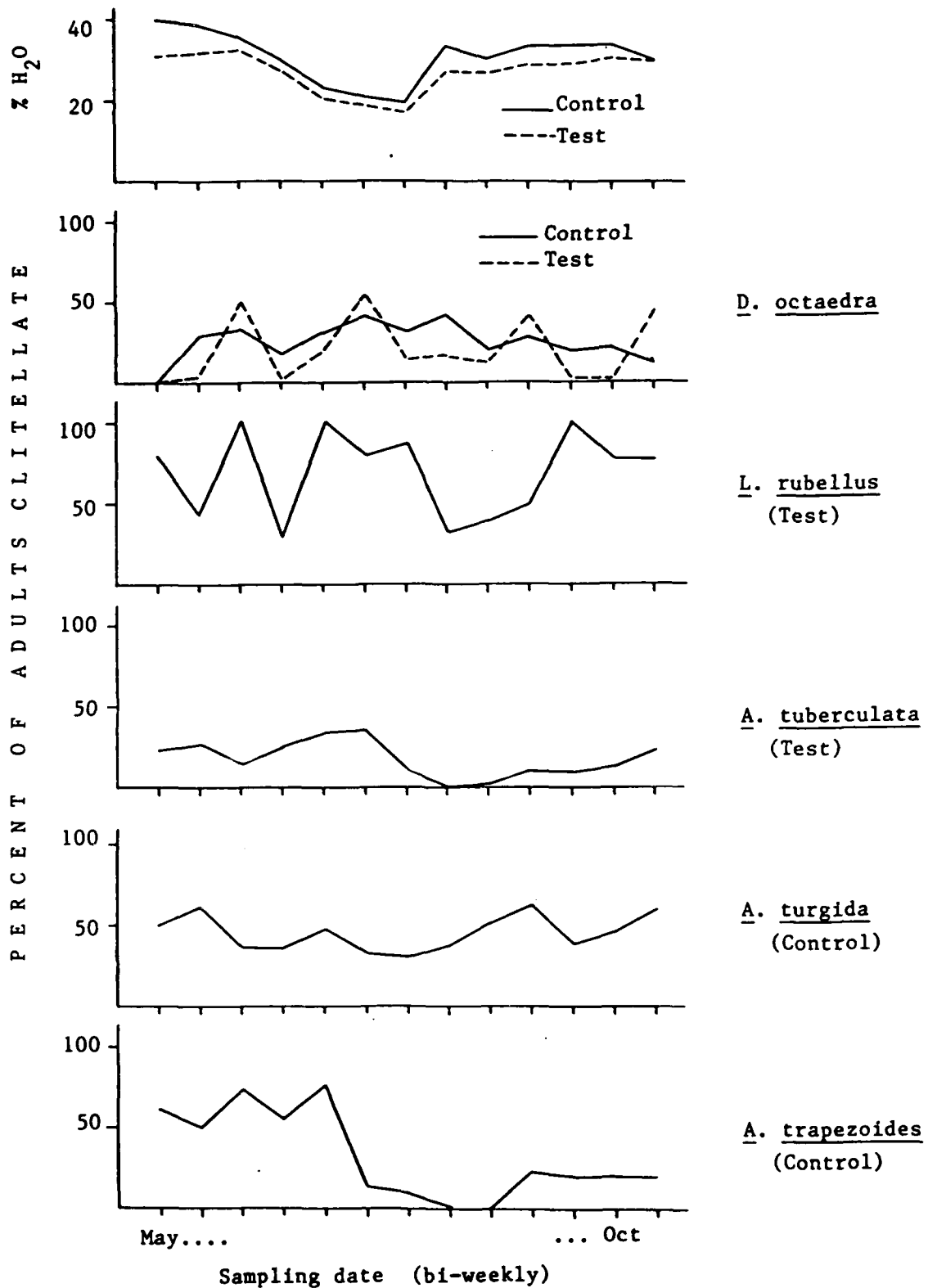


Fig. 29 . Percent moisture of A horizon (uppermost graph) and percent of adults in the clitellate state, for Test and Control lumbricids, 1985.

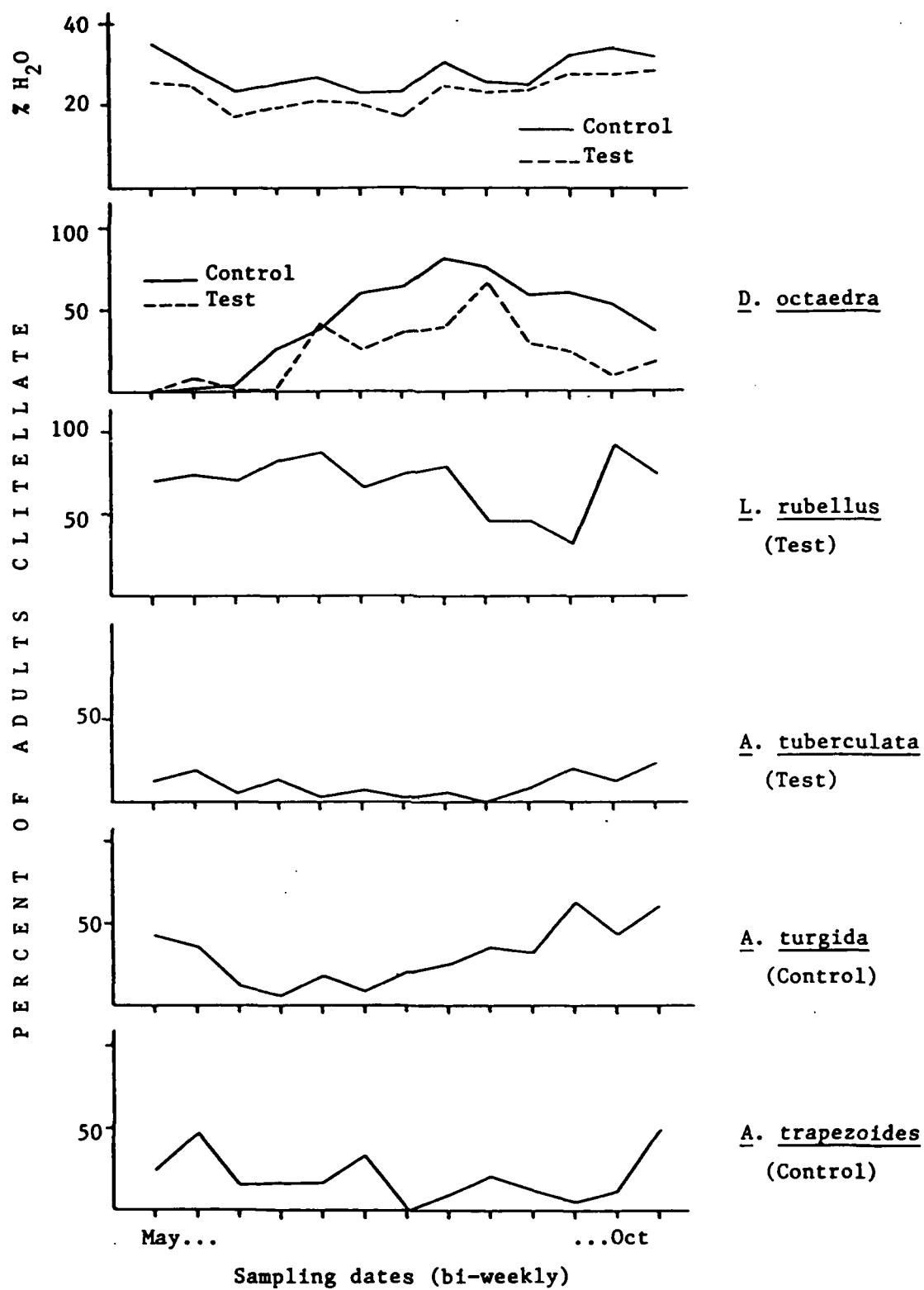


Fig. 30 . Percent moisture of A horizon (uppermost graph) and percent of adults in the clitellate state, for Test and Control lumbricids, 1986.

and thus reduce the explanatory power of regression analyses.

Efforts to untangle and quantify these relationships statistically are in progress. At this time, reproductive trends can only be used as a qualitative tool for interpreting fluctuations in other population parameters.

6. Cocoons

Cocoons obtained in 1984 through 1986 have now been examined, re-counted and weighed. Accuracy of stage determination was increased by first cleaning adhering soil from them. With only a small margin of error, three categories of development could thus be established without dissecting the cocoons:

a) new cocoons, with no sign of a developing embryo, often characterized by light-colored, translucent cases;

b) intermediate cocoons, portions of the developing worm visible between masses of nutritive material;

c) old cocoons, harboring a well-developed earthworm and little or no nutritive material.

6.1. Cocoon weights

For D. octaedra, we show mean mass of cocoon developmental stages in Table 24 (1985 data used as example). Old cocoons exhibited slightly lower mass than younger stages, a general trend also observed in other species. Because of frequently low replication, mass of intermediate and old cocoons may be of little statistical value in this project (although their numbers or frequencies are). Of prime importance, however, is the weight of new cocoons, for reasons discussed below. Yearly mass averages of new cocoons of abundant Test and Control species are summarized in Table 25.

Table 24. Yearly mean mass of individual cocoons of D. octaedra, by developmental stage, for 1985; means and ranges in mg.

	TEST	CONTROL
STAGE:		
NEW		
mean \pm SD	3.52 \pm 0.496	3.53 \pm 0.476
(N)	(180)	(717)
range	1.40 - 5.00	1.90 - 5.70
INTERMEDIATE		
mean \pm SD	3.51 \pm 0.470	3.62 \pm 0.449
(N)	(235)	(740)
range	1.90 - 4.90	1.90 - 5.40
OLD		
mean \pm SD	3.40 \pm 0.301	3.42 \pm 0.376
(N)	(30)	(93)
range	2.80 - 4.00	2.40 - 4.60

Table 25. Average yearly mass of individual new cocoons of Test and Control lumbricids (N cocoons weighed in parentheses).

	Means \pm SD, mg		
	1984	1985	1986
<u>D. octaedra</u> (C)	3.55 \pm 0.476 (772)	3.53 \pm 0.476 (717)	3.48 \pm 0.429 (673)
<u>D. octaedra</u> (T)	3.48 \pm 0.462 (307)	3.52 \pm 0.496 (180)	3.44 \pm 0.488 (241)
<u>L. rubellus</u> (T)	9.40 \pm 1.642 (322)	9.49 \pm 1.617 (400)	9.44 \pm 1.668 (460)
<u>A. tuberculata</u> (T)	21.52 \pm 4.075 (193)	19.59 \pm 4.169 (137)	19.36 \pm 4.940 (44)
<u>A. turgida</u> (C)	11.79 \pm 1.770 (174)	11.90 \pm 1.926 (181)	12.07 \pm 1.794 (129)
<u>A. trapezoides</u> (C)	24.91 \pm 3.436 (160)	24.79 \pm 3.536 (125)	24.65 \pm 3.229 (79)

Cocoon weights were distinctive for each species and varied little from year to year (Table 25). Preliminary t-tests showed that mean mass did not differ significantly between years in any species except A. tuberculata: cocoons were significantly heavier ($P < 0.05$) in 1984 than in either 1985 or 1986. We do not know for certain whether soil moisture influenced cocoon weights, or if so, whether it affected the cocoons themselves or the adults of A. tuberculata which produced them. The latter interpretation is, however, the more likely.

Cocoon weights are closely related to adult body mass (Lavelle 1981; Phillipson and Bolton 1977), but this relationship can only be established by monitoring individual earthworms and their cocoons. Field-collected data are insensitive in this case. Clitellate A. tuberculata, for instance, may range from <400 mg to >900 mg in any one year, and yearly mean mass varies without apparent relation to edaphic factors. Scant available information suggests that in addition to intrinsic variability, food availability and individual age may affect onset of sexual maturity at a given adult weight (Nowak 1975; Phillipson and Bolton 1977). In general terms, cocoon weights can thus be taken as indicators of the physiological state of adults.

Based on variances observed to date, estimates of detectable cocoon mass differences between sites or years range from $\leq 3\%$ for D. octaedra and L. rubellus to approximately 8% for A. tuberculata, at the 0.01 level of significance. Pending ANOVA of site and year effects, we come to the preliminary conclusion that cocoon weights furnish one of the most sensitive data bases for ELF lumbricids.

Statistical treatment

The cocoon weight data base currently consists of over 10,000 individual weights, classified by site, date, species and developmental stage. We have

so far not found any useful covariates for analysing yearly mean mass of cocoons. ANOVA of year effects (year and site effects in the case of D. octaedra) will thus be the main method employed.

6.2. Cocoon production

The ratio of mean yearly density of clitellate adults to mean yearly density of new cocoons is given in Table 26. Species which live near the soil surface or in litter generally produce much higher numbers than endogeics (Satchell 1967; Bouché 1972). In our sites, D. octaedra and L. rubellus accordingly lead in terms of cocoon production.

Table 26. Ratio of new cocoon : clitellate adult densities, based on yearly averages, for Test and Control lumbricids.

	$(N/m^2 \text{ new coc}) / (N/m^2 \text{ clit.})$		
	1984	1985	1986
<u>D. octaedra</u> (C)	9.40	6.19	4.52
<u>D. octaedra</u> (T)	6.89	7.84	5.74
<u>L. rubellus</u> (T)	4.41	4.22	4.30
<u>A. tuberculata</u> (T)	0.72	0.89	0.50
<u>A. turgida</u> (C)	0.72	0.82	0.75
<u>A. trapezoides</u> (C)	1.47	1.34	1.01

There is some evidence (Table 26), in epigeics as well as endogeics, that reduced proportions of reproductive adults (Table 23) in dry years coincide with fewer cocoons produced per adult. The relative resistance of A. turgida and L. rubellus to drought, discussed earlier, is again evidenced by stable cocoon production estimates (Table 26).

Values given in Table 26, we must point out, are useful for relative

comparisons only. They are underestimates of actual fecundity, since:

- a) yearly mean densities of cocoons do not reflect total production; and
- b) mean densities of clitellates do not account for the length of time adults remain reproductive, and thus would be "counted more than once" in field samples. We are currently investigating means of obtaining more accurate fecundity estimates.

6.3. Cocoon density, development and emergence

Seasonally and yearly, densities vary as a function of new cocoons produced and old cocoons hatching, and are also influenced by climatic variables (via the physiological state of adults). Resulting emergence and population dynamics patterns may thus vary between years.

Figs. 31-32 show cocoon and hatchling densities for D. octaedra in Test and Control, summarized by month. Typically, a peak in old cocoons occurs in the spring, followed by high densities of small immatures in June and July. What appeared to be continued recruitment in August of 1985 (Fig. 31) was likely a result of delayed growth due to mid-summer drought. This interpretation is in agreement with observations by Rundgren (1977) in Sweden. A similar phenomenon was recorded in the Test site for A. tuberculata (Fig. 33), a species which also exemplifies the potential year-to-year variability in cocoon development and hatching.

High numbers of new cocoons were produced by A. tuberculata in late 1984. They overwintered, matured in May and June of 1985, and produced large numbers of hatchlings in July and August. These immatures may then have become inactive, concurrent with virtual cessation of reproduction by adults. Small immatures were thus carried over into the 1986 population (fully developed cocoons were absent in fall 1985 and spring 1986). In early 1986, they again encountered severe moisture stress and retarded growth (Fig. 33).

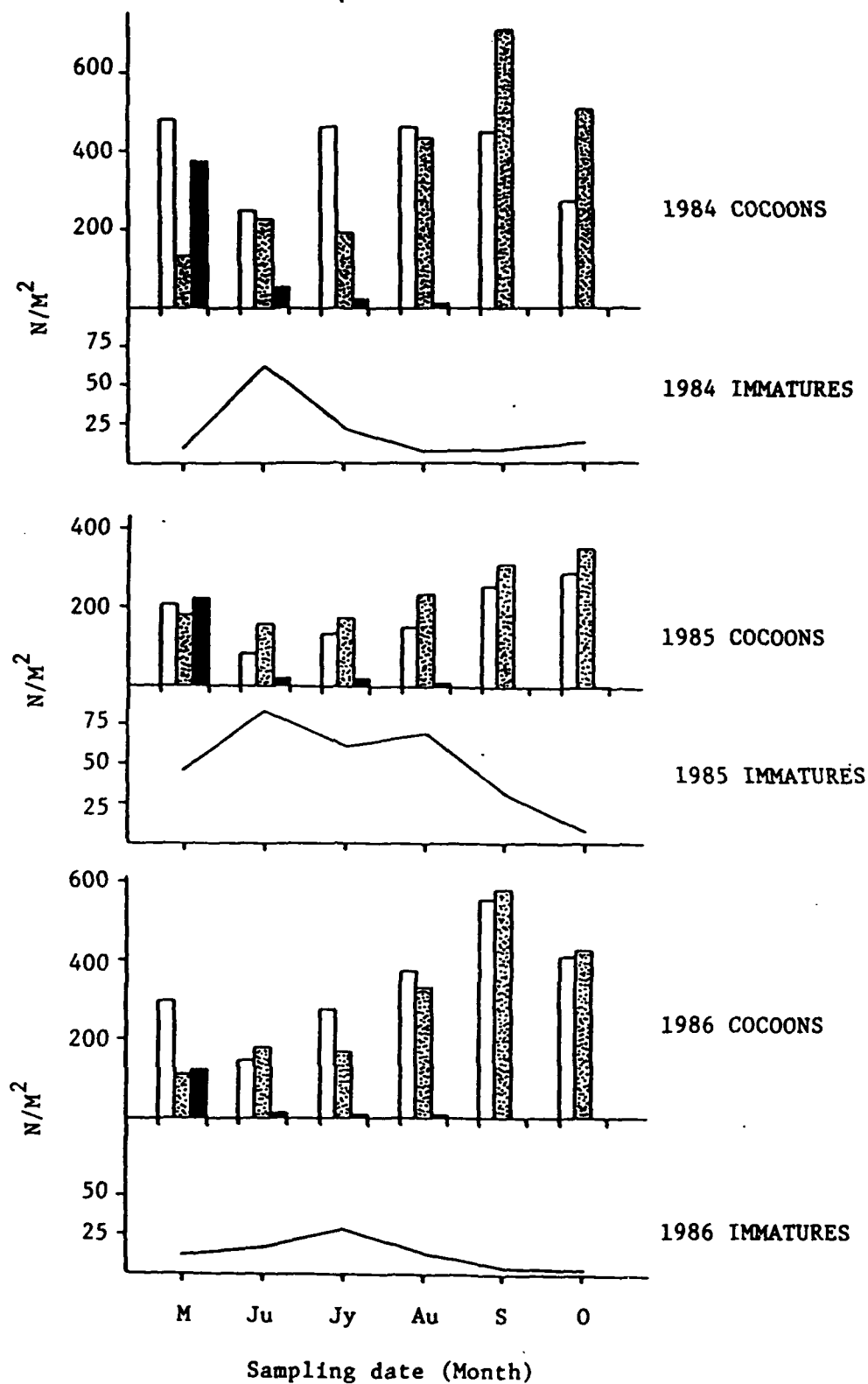


Fig. 31 . Densities of new, intermediate and old cocoons (open, dotted and black bars respectively), and of small immatures (≤ 6.0 mg) of *D. octaedra* in Control, 1984-86.

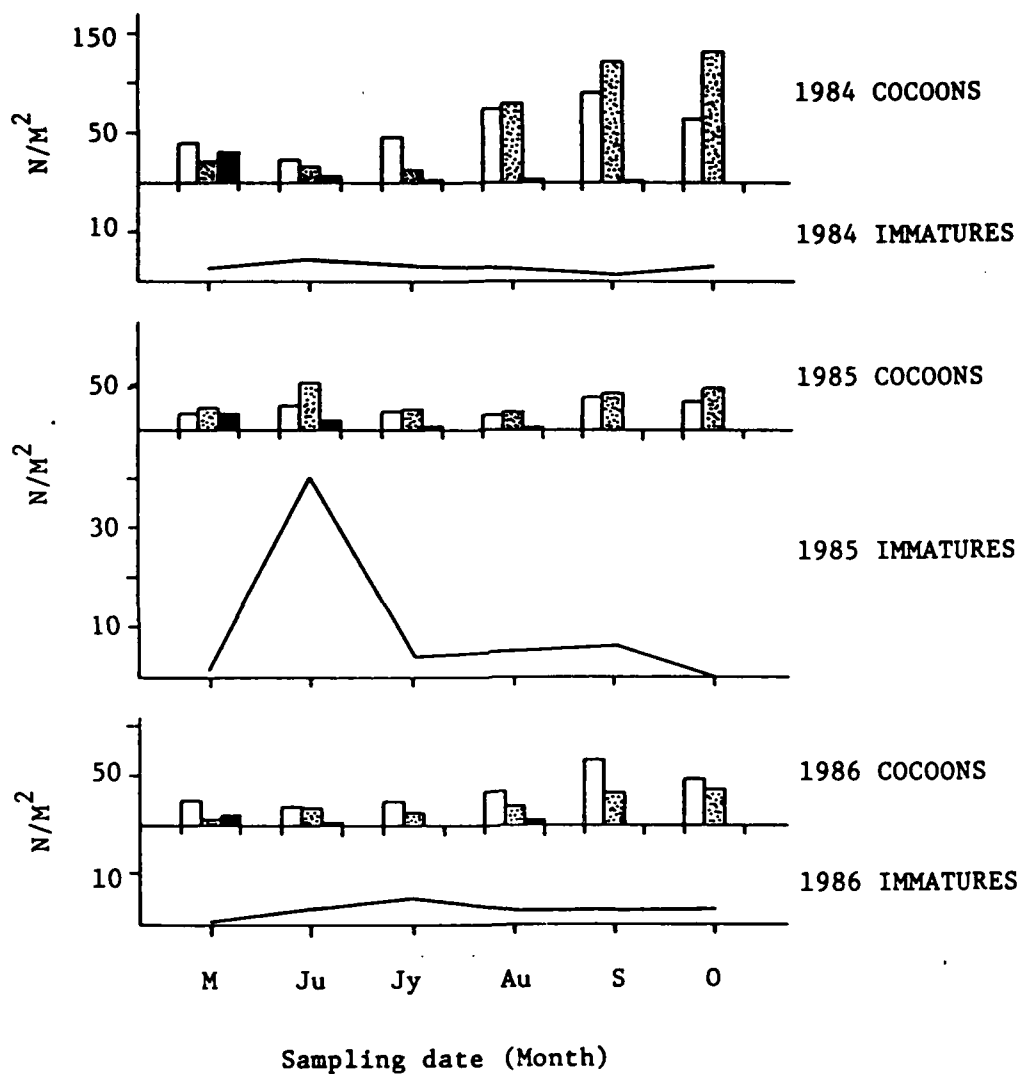


Fig. 32 . Densities of new, intermediate and old cocoons (open, dotted and black bars respectively), and of small immatures (≤ 6.0 mg) of D. octaedra in Test, 1984-86.

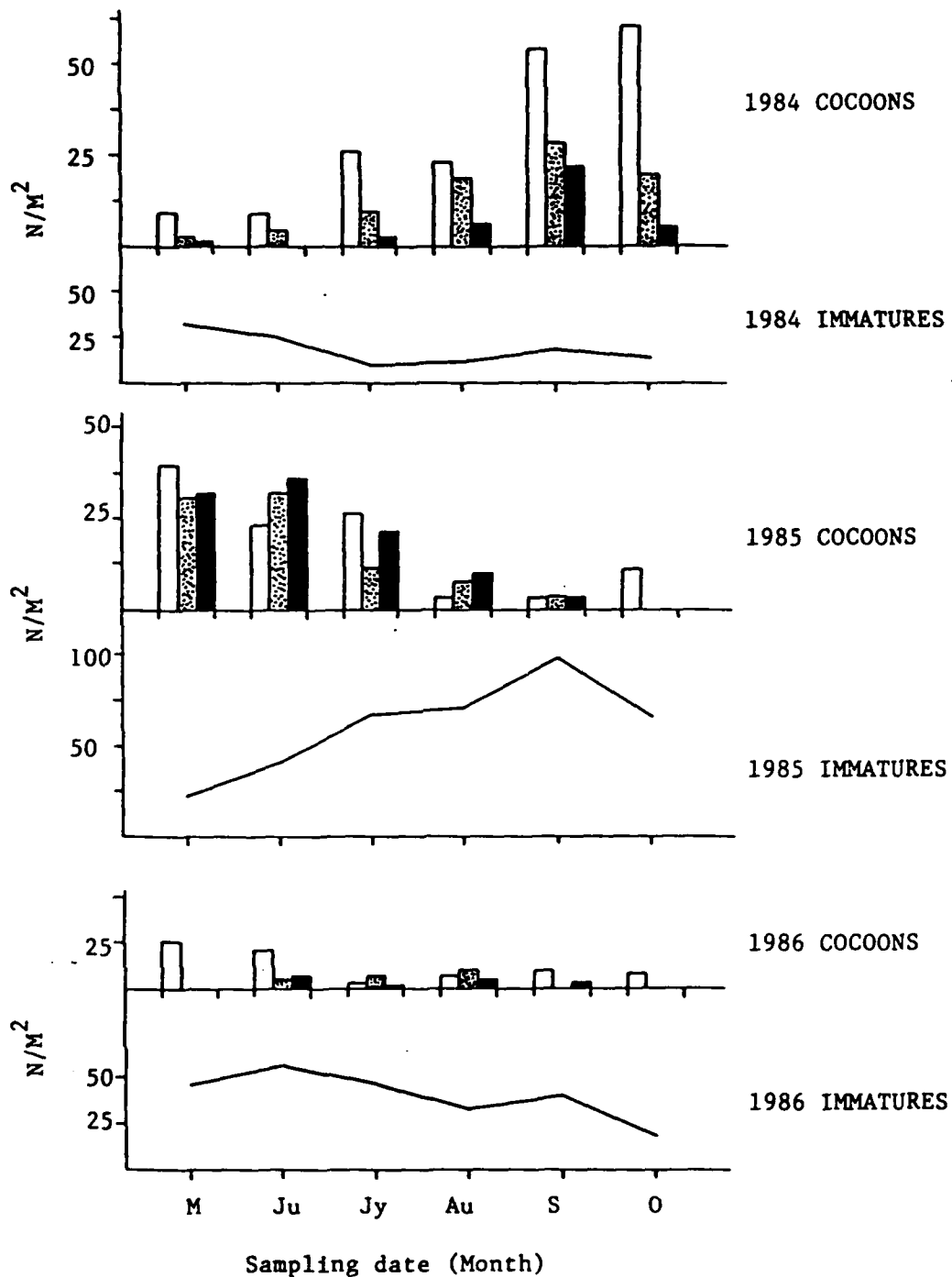


Fig. 33 . Densities of new, intermediate and old cocoons (open, dotted and black bars respectively), and of small immatures (≤ 30 mg) of *A. tuberculata* in Test, 1984-86.

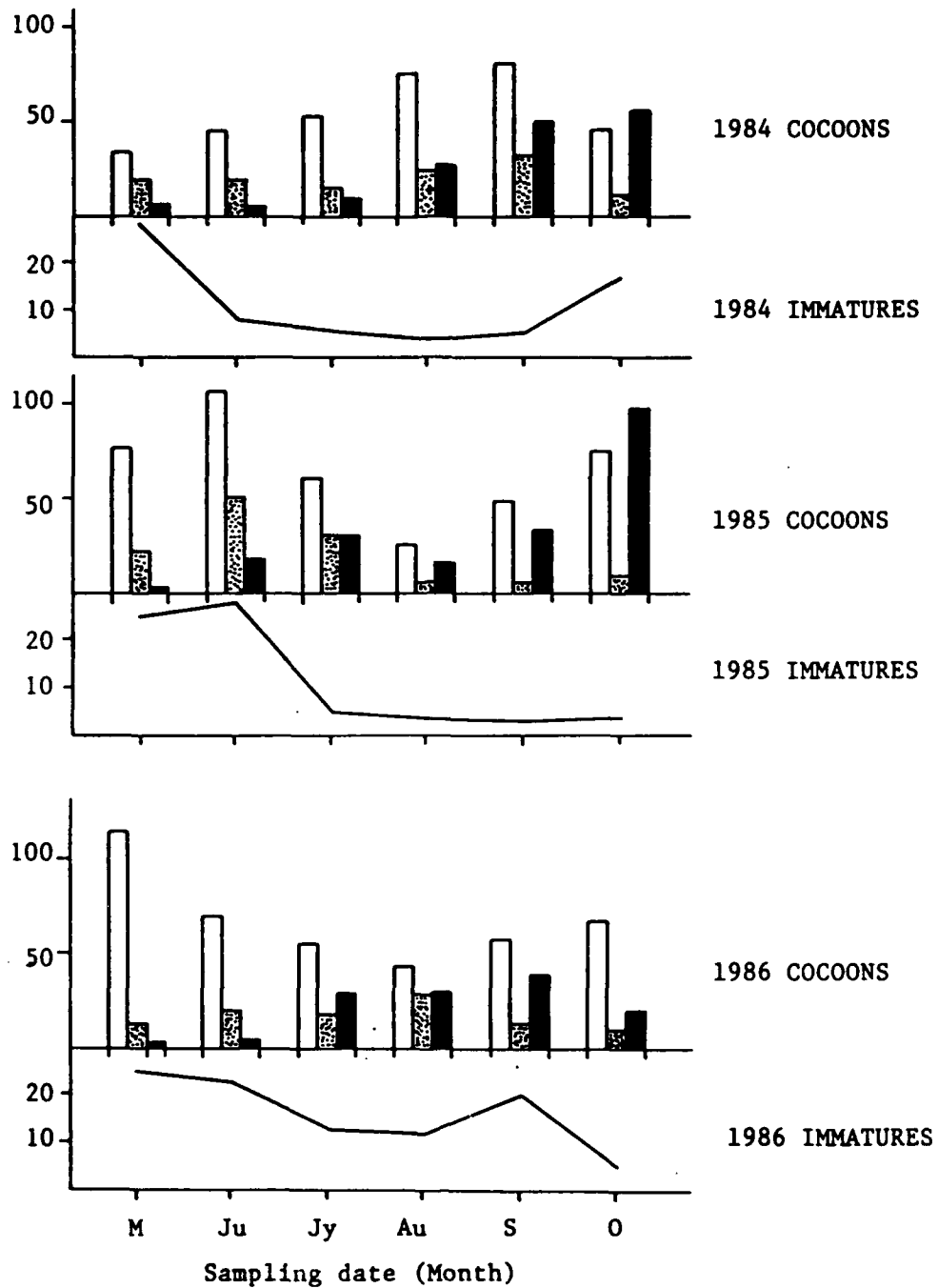


Fig. 34 . Densities of new, intermediate and old cocoons (open, dotted and black bars respectively), and of small immatures (≤ 20 mg) of L. rubellus in Test, 1984-86.

Yearly patterns of recruitment were much less variable in L. rubellus (Fig. 34). Old cocoons accumulate in the fall, small immature density peaks in May and June, with a lesser peak possible in the fall. New cocoons are produced at any time of year, subject to some modulation during dry periods (e.g., July-August 1985).

In general, data on all species concur with observations by Nowak (1975) and Rundgren (1977). Cocoons produced early in the year hatch later the same year. Summer and fall cocoons overwinter and hatch the following spring. Appearance of small immatures follows the disappearance of old cocoons from samples, but emergence patterns must be interpreted with caution if individuals tend to become inactive at low soil moistures.

Dendrobaena octaedra showed the most consistent pattern of cocoon development from year to year. Summed by month, relative frequencies (%) of new, intermediate and old cocoons were therefore tested with respect to site and year effects (four-way contingency analysis: 2 sites, 3 years, 6 dates, and 3 developmental classes; Cochran-Mantel-Haenszel statistics available by SAS).

Year effects were highly significant, i.e., we can expect between-year differences in cocoon stage frequencies. It is apparent from Figs. 31-32 that unusually high or low production of new cocoons will produce this effect.

Association between sites and stages, however, taking years into account, was not significant ($P > 0.05$); i.e., the relative frequencies of cocoon stages over the seasons did not differ between sites.

Despite differing densities of cocoons, developmental patterns thus provide quantifiable tools for between-site testing of D. octaedra.

Dendrobaena octaedra: a summary:

We can now show the long-term consequences of reproduction and recruitment in D. octaedra populations (Fig. 35).

Large numbers of cocoons were produced in 1984, the entire adult population being reproductive in the second half of the season (Fig. 28). Immature abundance in 1985 was greatly increased as a result, but only a small proportion of adults was able to reproduce, leading to low cocoon numbers in 1985. Individuals immature in 1985 then swelled the adult contingent in 1986; although the proportion of clitellates was low (relative to 1984), higher absolute numbers of them were able to increase cocoon densities (relative to 1985) in spite of early-season drought (Fig. 35).

One year's reproductive peak was thus expressed in increased adult numbers two years later. High densities of immatures in the intervening year of 1985 furnish the bridging evidence for our conclusion that the species takes approximately two years to mature.

Although this pattern was similar in Test and Control, the relative number of immatures was discrepant in 1986 (Fig. 35). It is possible that soil moisture, generally lower in Test, retarded maturation, keeping higher numbers of large-bodied individuals in the immature state during this year (1986) of unusual moisture stress. In the following section, this interpretation will be supported by means of weight class analysis.

7. Weight class distribution

Here, attainment of a given body mass as a result of growth is the main parameter for analysis, while development of external sexual characters can be ignored. If growth rates are similar in Test and Control, seasonal frequencies of individual mass should not differ greatly (even if, as

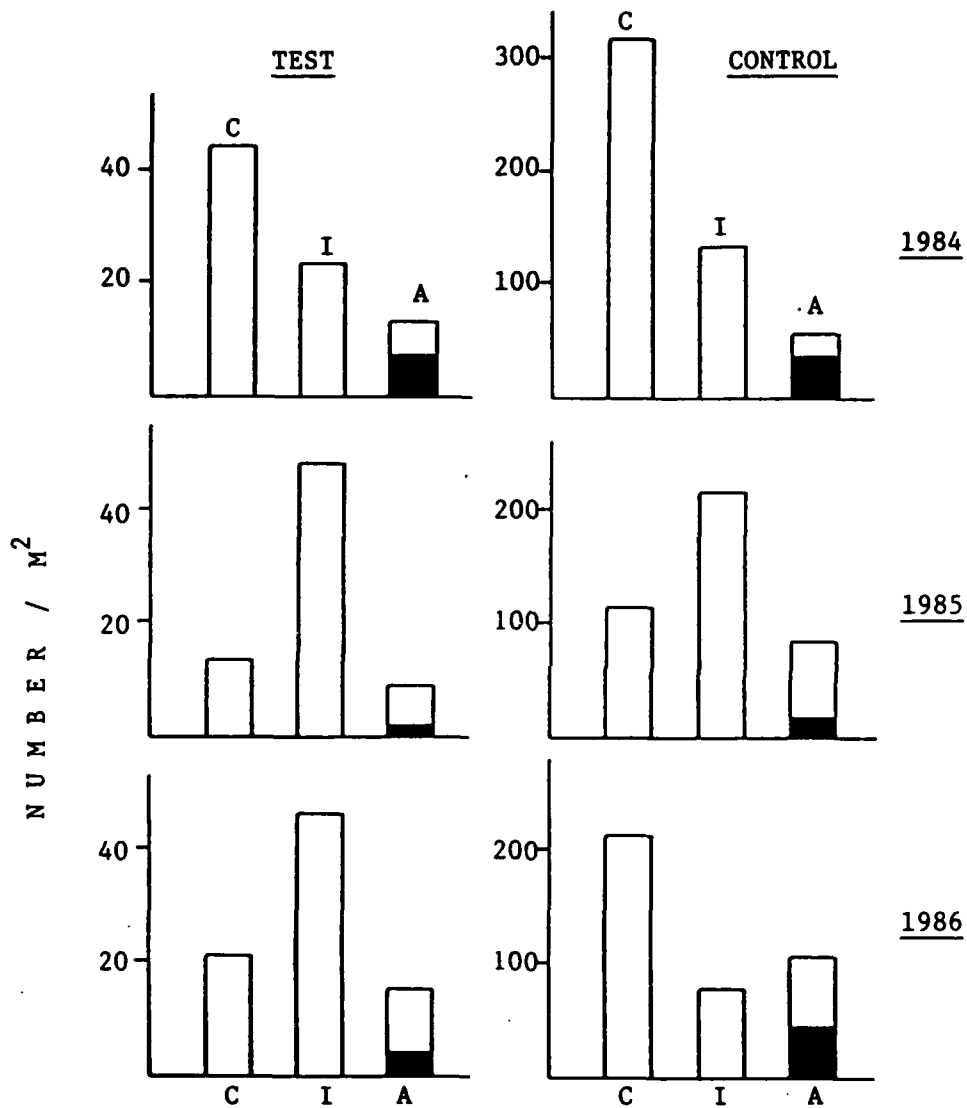


Fig. 35. Yearly mean densities of new cocoons (C), immatures (I) and adults (A) of *D. octaedra* in Test and Control, 1984 - 1986.

Black portion of bars = densities of clitelate adults.

suggested above, large immatures in Test may remain immature while large immatures in Control become clitellate adults).

Data on D. octaedra in Test and Control were tested for discrepancies in weight class frequencies by contingency tables: year effects by means of 3 x 6 tables (3 years, each summed over sites, 6 classes); and site effects by 2 x 6 tables (2 sites, each summed over sites, and 6 classes).

Frequency distributions were found to be equal between sites ($P < 0.005$), indicating that the general patterns of recruitment and growth furnish a good indicator for site comparison.

However, while contingency tables are useful for general comparisons, any "lumping" must be used with caution. If ELF should affect lumbricids, it may:

- a) affect developmental classes differentially;
- b) affect the same class differently at different times of year; and
- c) be gradual and therefore not detectable if 1st to 3rd operational years are combined.

Statistical treatment

The computerized data base now consists of close to 17,000 individual worm weights for just the three species of main interest (D. octaedra in Test and Control, and L. rubellus and A. tuberculata in Test). Not all of it has been appropriately formatted as yet, but we expect completion of pre-ELF analysis in mid-1988. Two basic ANOVA models are considered:

- a) For single species, single site, multi-year analyses, e.g., of Test lumbricids:

Sources of variation: Years
 Samples/year (Error 1)
 Dates
 Years x Dates
 Samples x Dates (Error 2)

Significant Year x Date interaction would indicate that frequency distribution shifted seasonally from one year to another, leading to closer examination of individual treatment cells.

b) For D. octaedra in Test and Control, multi-year comparison:

Sources of variation: Sites
 Samples/site (Error 1)
 Years
 Sites x Years
 Samples/year (Error 2)
 Dates
 Sites x Dates
 Samples/date (Error 3)
 Years x Dates
 Sites x Years x Dates
 Samples x Years x Dates (Error 4).

NOTE: To both models, the following applies:

--- The data base consists of % in each weight class (of all worms/sample), using arcsine transformation as appropriate for percentages (Sokal and Rohlf 1981);

--- "Samples" = either the percent worms in a single weight class of interest, e.g. small immatures or adults; or,
 "samples" = a vector of weight classes which are tested as a multivariate array. Both kinds of variates are thought important to test because of the potentially differential effects of ELF on different segments of the population.

VI. LITTER INPUTS AND DECOMPOSITION

1. Litter inputs

Much as in previous years, litterfall was synchronous in Test and Control in 1987, abscission of maple leaves peaking in the weeks of September 22 to October 7. Table 27 provides a summary of yearly inputs/m², excluding small debris. ANOVA showed neither significant year nor site effects ($P > 0.3$).

Table 27. Yearly inputs of maple and total leaf litter /m² (N= 20 traps/site).
(T = Test, C = Control).

		A v e r a g e g d r y / m ² ± SE									
		1983		1984		1985		1986		1987	
		T	C	T	C	T	C	T	C	T	C
Maple		189.0 ±14.5	218.3 ±12.0	175.3 ±12.4	179.0 ± 9.0	203.5 ±14.3	198.6 ± 8.6	176.0 ±13.2	189.1 ±11.5	160.9 ±14.5	180.0 ±12.6
Total		278.2 ±13.2	304.9 ±10.8	259.2 ± 8.8	264.0 ± 7.4	285.6 ± 7.3	288.6 ± 4.9	251.6 ± 7.2	284.2 ± 9.7	231.2 ± 9.1	274.3 ± 8.6

2. Litter standing crops

Even with 40 samples/ date, standing crop estimates have been relatively variable over the seasons in both sites. In general, standing crops tended to be higher in Control (Fig. 36). ANOVA of site and year effects is pending (files consist of over 3,000 data points and have been formatted for analysis).

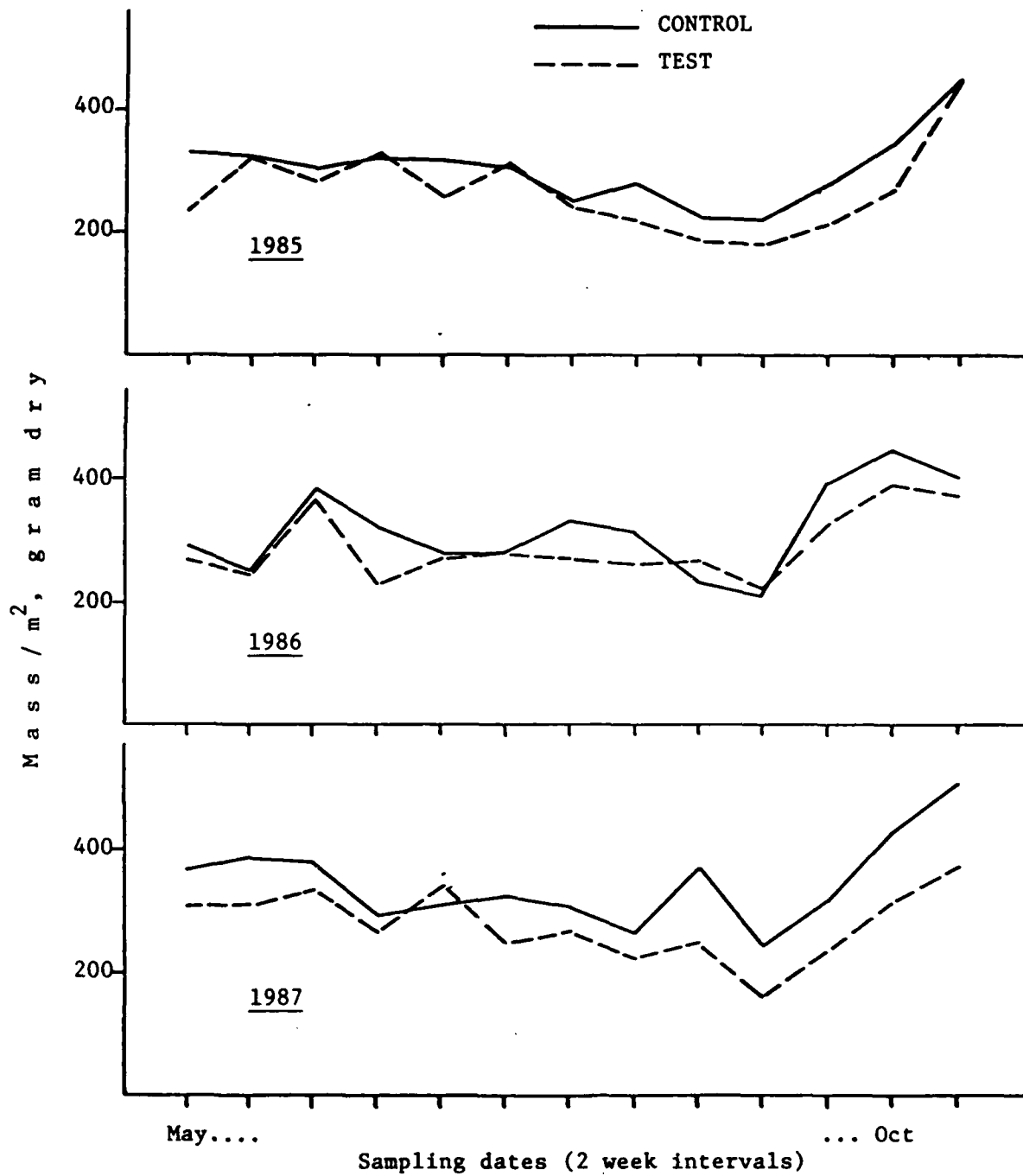


Fig. 36. Average litter standing crops in Test and Control, 1985-87
(N = 40 per date and site).

3. Turnover rates of forest floor litter

Exponential decay rates of forest floor litter are calculated based on total annual litter input and maximum standing crop after leaf fall (Olson 1963).

Much of the fine debris collected in litter traps consists of frass and feces. Leaf fragments, even if small, are usually identifiable as to species, based on venation, texture and peripheral serration (and are therefore included in maple, basswood, or other categories).

On the other hand, non-leafy debris, e.g. flowers from the preceding spring, tends to be excluded from samples of standing crops taken in the field. For estimates of decay rates, we therefore used total litter inputs minus debris, and maximum October standing crop values.

Loss rates did not differ significantly between sites, and average turnover time for leaf litter was approximately one year (Table 28). Because of the sources of error mentioned above, these are likely to be underestimates. Real turnover time is probably more than one year, the remainder of one year's leaf fall completing decay during its second fall and winter on the forest floor.

Estimated turnover times obtained in 1987 (Table 28) were higher than in previous years for Test as well as Control. Slightly lower total litter inputs (Table 27) may have affected these results: in both sites, we observed an unusual degree of herbivory by geometrid larvae in early summer.

Table 28. Loss rates and turnover times for forest floor leaf litter in Test and Control. In parentheses: upper and lower 95% confidence limits, calculated from upper and lower limits on inputs and standing crops.

	Exponential loss rate k	Turnover time (years) 1/k
Test 1985	-1.01 (-1.09 to -0.95)	0.99 (0.92 to 1.05)
Test 1986	-1.04 (-1.07 to -1.01)	0.97 (0.94 to 0.99)
Test 1987	-0.82 (-0.88 to -0.77)	1.22 (1.13 to 1.30)
Control 1985	-1.03 (-1.16 to -0.94)	0.97 (0.86 to 1.06)
Control 1986	-1.00 (-1.01 to -0.99)	1.00 (0.99 to 1.01)
Control 1987	-0.78 (-0.84 to -0.74)	1.28 (1.20 to 1.36)

4. Litter mass loss

The litterbag method has been used by many investigators to study decomposition in terrestrial ecosystems (e.g., Witkamp and Olson 1963; Witkamp and Crossley 1966; Cromack and Monk 1975; McClaugherty et al. 1985). In our sites, we have used this method as well as variations of it (leafpacks) in order to quantify mass loss of maple litter. In the following, we summarize the main results obtained by different methods and discuss their value for future Test-Control comparison.

Litterbag studies (1 and 5 mm monofilament nylon mesh) were begun in November 1984; a pilot series of leafpacks (6 leaves/pack, of known individual weights and areas) was initiated at the same time, in three

sets: sun, shade and randomly mixed leaves. Replication was limited, and they will be disregarded here in favor of the more rigorously controlled second leafpack series. The latter consisted of sun and shade leaves only, and was initiated in November 1985. At the same time, litterbags with mesh size approx. 20 mm were placed in the field.

For site comparison, we may examine first-year mass loss data for litterbags and leafpacks, as well as % mass remaining after a second winter in the field (approx. 18 months) (Table 29). With the single exception of mixed leafpacks after 18 months exposure, mean mass remaining did not differ between sites for any one method employed. Although 5 mm litterbags allowed more rapid decomposition than 1 mm mesh bags, both retarded mass loss when compared to the 20 mm bags used in 1985 (Table 29). Loss of material during retrieval of 20 mm bags had negligible influence on these data (and is likely to be compensated by small debris which cannot be separated from the decomposing leaves).

After 12 months exposure, litter in 20 mm bags retained slightly less than 50% of initial mass, comparable to sugar maple decay rates in southern Wisconsin (McClaugherty et al. 1985). Mixed leafpacks (84) experienced mass losses close to that in large-mesh bags (85), excepting the unexplained outlying value for leafpacks in Control. As expected (Heath and Arnold 1966; Herlitzius and Herlitzius 1977), shade leaves decomposed to a much greater degree than did sun leaves (Table 29).

For the major decomposition series, decay constants were estimated by semi-log regression (Olson 1963), which allows us to test regressions between sites (Table 30). All regressions were significant at $P < 0.001$, with coefficients ranging from 0.40 to 0.85. The latter were thus relatively low, but regression slopes did not differ between sites (within each series), at $P > \text{or } \gg 0.05$. We noted a single exception: 5 mm bags ($P < 0.05$).

Table 29. First-year and 18-month decay of maple litter, in % of initial mass, for litterbags and leafpacks. In parentheses: year of initiation. Values which do not include confidence limits are final weights after 12 months, estimated by regression.

	Mean % remaining mass \pm 95% CL	
	TEST	CONTROL
Litterbags 1 mm (84)		
12 months	69.7	67.0
18 months	55.1 \pm 2.25	56.6 \pm 2.90
Litterbags 5 mm (84)		
12 months	52.4	57.6
18 months	43.5 \pm 4.17	43.1 \pm 1.28
Mixed leafpacks (84)		
12 months	50.2	47.2
18 months	34.4 \pm 4.72	44.4 \pm 3.58 *
Litterbags 20 mm (85)		
12 months	47.3	49.8
18 months	35.8 \pm 4.72	37.5 \pm 3.29
Sun leafpacks (85)		
12 months	61.6 \pm 2.20	59.2 \pm 4.38
18 months	51.3 \pm 7.87	57.4 \pm 8.80
Shade leafpacks (85)		
12 months	24.8 \pm 2.21	26.6 \pm 3.50
18 months	24.5 \pm 4.56	26.2 \pm 3.92

* Means in the same row differ at $P < 0.05$.

Table 30. Semi-log regressions for first-year litter decay in Test and Control. Year of initiation in parentheses. X= months; final mass at 12 months estimated for all but leafpacks; $k = -\ln(X_t/X_0)$; $1/k$ = years.

			r	k	1/k
Litterbags 1 mm (84)					
Test	lnY= 4.4658 - 0.0185X	-0.48	-0.36	2.78	
Control	lnY= 4.5129 - 0.0257X	-0.76	-0.40	2.50	
Litterbags 5 mm (84)					
Test	lnY= 4.7238 - 0.0638X	-0.78	-0.65	1.54	
Control	lnY= 4.6038 - 0.0459X	-0.85	-0.55	1.82	
Litterbags 20 mm (85)					
Test	lnY= 4.5483 - 0.0577X	-0.70	-0.75	1.33	
Control	lnY= 4.3259 - 0.0347X	-0.40	-0.71	1.41	
Leafpacks shade (85)					
Test	lnY= 4.4356 - 0.0971X	-0.81	-1.39	0.72	
Control	lnY= 4.5072 - 0.1024X	-0.83	-1.33	0.75	
Leafpacks sun (85)					
Test	lnY= 4.6966 - 0.0495X	-0.77	-0.48	2.08	
Control	lnY= 4.6759 - 0.0465X	-0.81	-0.52	1.92	

Gosz et al. (1973) calculated a decay parameter of $k = -0.51$ for sugar maple at Hubbard Brook, which is lower than those derived from our large-mesh bags (85) ($k = -0.75$ and -0.71 for Test and Control respectively). Given that decay rates vary with latitude (summary in Louisier and Parkinson 1976) as well as with site characteristics (Boerner 1984; Herlitzius 1983), our estimates fall within the range of available data.

Values of k derived from 20 mm bags yielded turnover times of 1.3 to 1.4 years (Table 30). For forest floor litter (Table 28) $1/k$ was estimated as approx. one year. Actual rates probably lie between these estimates. On one hand, some of the previous year's litter is still present at the time of the next leaf fall; on the other hand, processing and distribution of litter-bags delays the onset of decomposition, at a time when freshly fallen litter undergoes initially rapid mass losses.

In general, we conclude that virtually any method would be appropriate for testing between-site differences in litter mass loss. Observed variation offers no consistent reasons for choosing one method over another: expressed in terms of confidence intervals, approx. 5 to 13% of the means seemed to be the general range. We favor large-mesh (20 mm) bags because they seem to yield data relatively close to biological reality, and are no more variable than others. Our choice seems justified in view of analytical results discussed below.

5. ANOVA of mass loss in large-mesh bags

For the 20 mm litterbag series initiated in 1985, ash-free dry weights were obtained for all samples. Note that data in Table 29 were not derived from ash-free dry weights, so that comparison with other decomposition data could be made.

First-year regressions of $\ln(\text{ash-free mass remaining})$ on time were highly significant for both sites, at $P < 0.0001$, and regression slopes did not differ ($P > 0.05$).

ANOVA of first-year mass loss data indicated that neither site effects ($P > 0.07$) nor site x date interactions ($P > 0.5$) were significant. Using data from the entire sequence (samples taken May 1986 through September 1987), ANOVA again showed no significant site effects ($P > 0.2$). Use of 20 mm bags, for biological as well as statistical reasons, thus appears indicated.

6. Analysis of single leaf mass loss

Leafpack studies were designed to quantify leaf-specific decay rates in Test and Control. The series initiated in November 1985 consisted of sun and shade leafpacks, and we were able to sample them through November 1986, for a full year data base.

While Tables 29 and 30 describe leafpack decay based on total pack mass, leaf-specific mass, surface area, and position in the pack were used in ANOVA in order to detect potential site effects.

Table 31 summarizes significance levels for main effects and interactions for both leaf types. As was expected (ref. only to columns A and B in Table 31), effects of date and position in the pack were highly significant. Site x position interactions were not significant: in both sites, the top leaves of shade packs, and the bottom leaves of sun packs, tended to decompose more rapidly than their lower or upper counterparts (Fig. 37). Moisture and contact with soil exerted a greater relative influence on sun leaves (loss of leaf portions by breakage was very minor during the first year, judging by final leaf shape vs. initial xerox images).

Table 31. Significance levels and sources of variation of ANOVA with one covariate (initial area/weight ratio) for sun and shade leafpacks (first-year decomposition).

Source of variation	d.f.	A	B	C
		SHADE	SUN	SHADE**
		P	P	P
Sites	1	0.04	0.25	0.32
Dates	7	0.0	0.0	0.0
Position	5	0.0001	0.0	0.0001
SitexPosition	5	0.42	0.34	0.42
SitexDate	7	0.0001	0.007	0.0001
DatexPosition	35	0.002	0.36	0.03
SitexDatexPosition	35	0.97	0.10	0.88
Ratio area/weight	1	0.007	0.0001	0.08
Error (Shade)	832			
Error (Sun)	851			

** NOTE: Column C = results of ANOVA without May 4, 1986 data.

The covariate (ratio area/weight) was also significant, particularly in the case of sun leaves; this was not unexpected, since that ratio reflects leaf structure and quality characteristics.

Interaction between sites and dates was evident in both leaf types, indicating that at some time during the year, decay rates varied between sites.

Regarding the most important factor, overall site effects were significant for shade leaves at $P = 0.04$ (column A in Table 31). Examination of original data (Fig. 38) identified the sample taken on May 4, 1986, as the single

outlying mean. The raw data showed the May samples from Control as having contained an unusual number of leaves with marginal area/weight ratios, i.e., leaning toward sun leaf characteristics. After eliminating May 4 data from the total array, ANOVA results (Column C, Table 31) now showed that site effects were no longer significant. Interestingly, the influence of area/weight ratios also lost its significance when the first sampling date was eliminated. Apparently this ratio is an important factor in decay of heavier, thicker leaves ($P = 0.0001$ for sun leaves), but not in true shade leaves. In the field, the latter rapidly lose much of their laminae, becoming vertically penetrable throughout a leafpack.

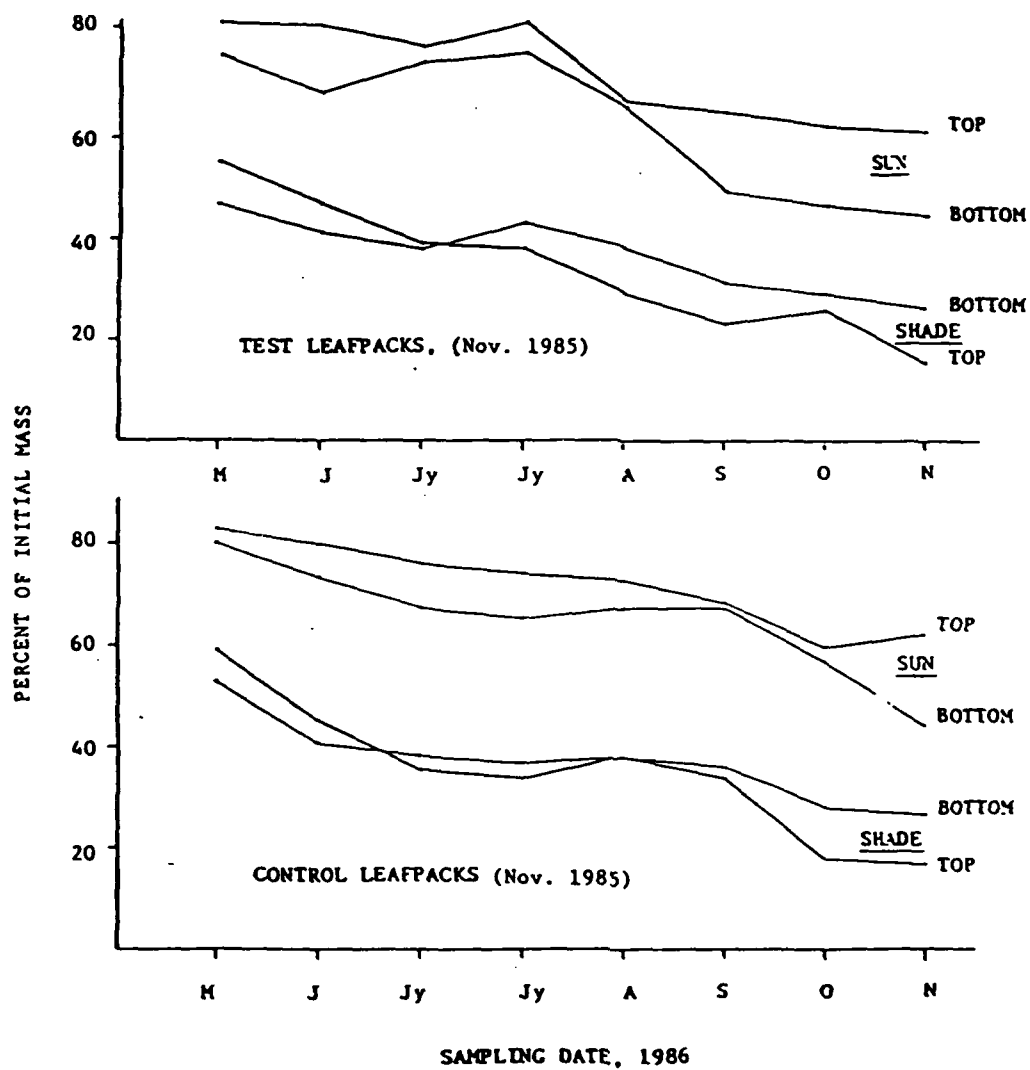


Fig. 37. Percent remaining of sun and shade leaves in top and bottom positions of Test and Control leafpacks placed in the field in November 1985.

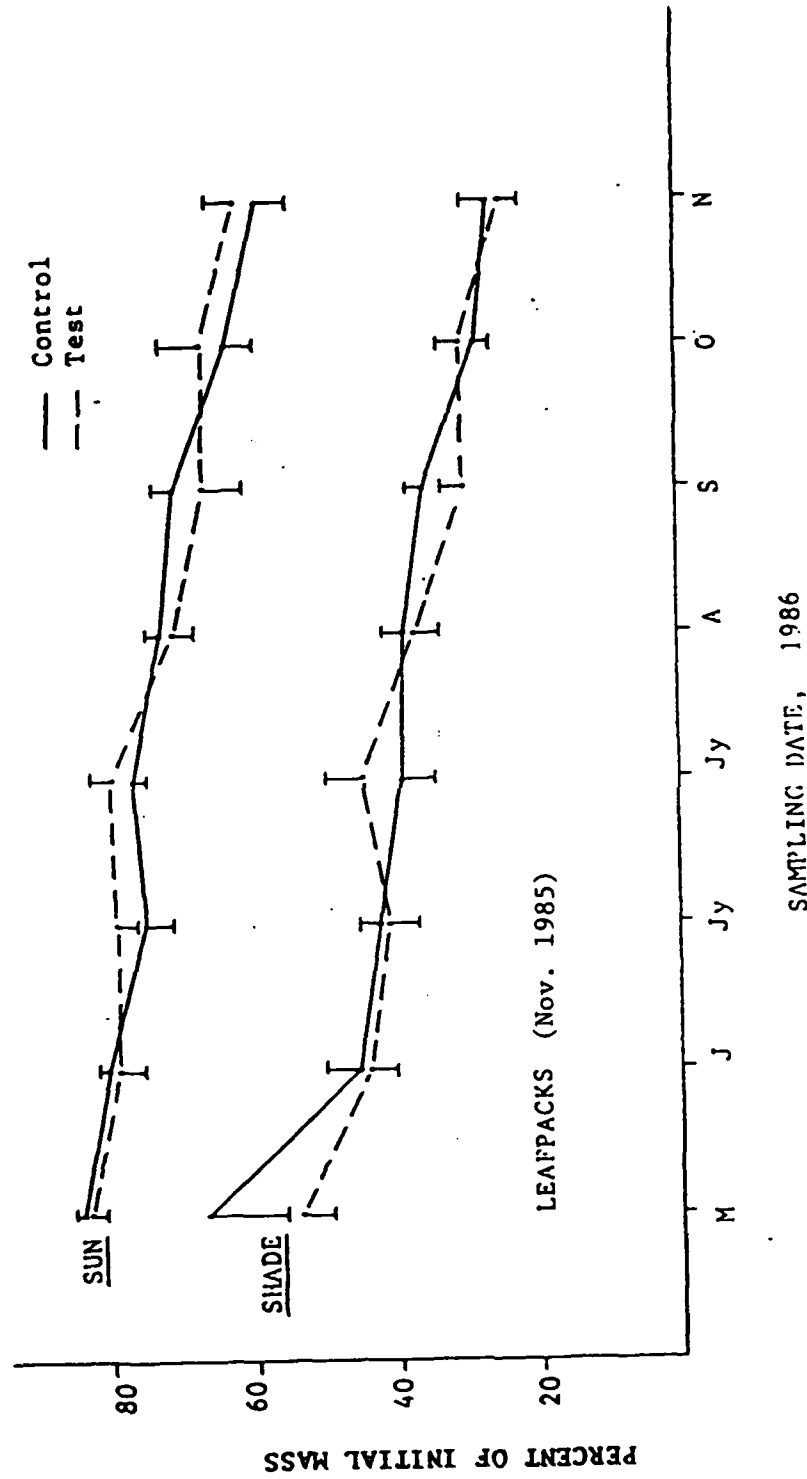


Fig. 38. Percent remaining (means \pm 95% CL) of sun and shade leafpacks placed in the field in November 1985.

7. Proposed work

Based on the foregoing, we propose that a single method for monitoring litter breakdown in Test and Control will suffice for detecting potential differences in decay rates. We selected 20 mm mesh (thin nylon netting) litterbags, as used in 1985. Although leafpacks yield more detailed data, their preparation and sampling is tedious, and they do not offer "better" data in terms of site-specific decay rates. Furthermore, November snowfall can prevent placement of leafpacks in the field (it very nearly did in 1985), because the time between abscission and onset of winter is marginally short for preparing and assembling packs.

Currently implemented and proposed schedules of large-mesh litterbag experiments are summarized in Fig. 39 .

This schedule will make the following comparisons possible:

1. First-year decay rates, pre-ELF vs. first and third ELF years;
 2. Second-year decay rates, pre-ELF vs. third and fourth ELF years;
 3. EXCHANGE series (Fig. 39): a replicate litterbag series for the 1990-1992 period, using Test litter in Control, and Control litter in Test litterbags.
- We are reserving this experiment for the last two years for the following reasons: ELF effects are likely to be subtle, but may accrue to a detectable point in later years. Even then, results of the routine litterbag series may be marginal or ambiguous. Concurrent monitoring of site-specific and exchanged litters will allow distinction between site- and ELF- effects, if any. Although 1992 is no longer a true field year, collecting litterbags at monthly intervals for processing at MSU will not be a problem.

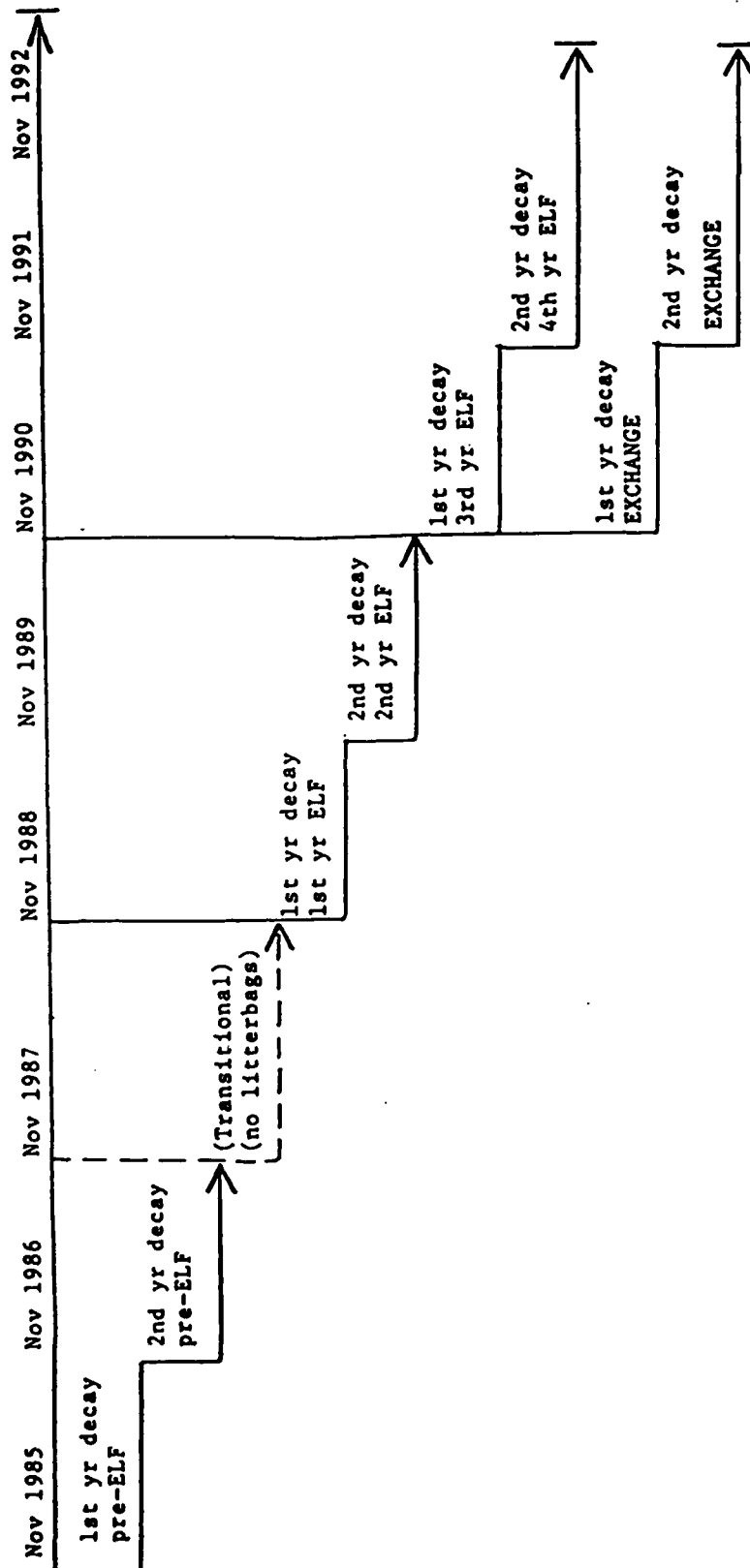


Fig. 39. Present (to November 1987) and proposed schedule of litterbag studies in Test and Control. For explanation of EXCHANGE see Text.

Statistical treatment

Mathematical description of the data, by year, will characterize observed mass loss over time. Semi-log regression (as above, after Olson 1963) will be used, to that regression parameters can be tested between sites and years.

Treatment (site) means will be compared by ANOVA, applied to one pre-ELF and two ELF series of litterbags (first- and second- year decay data separated): main factors sites, years, and dates, and interactions.

The last series of litterbags, using within-site litter as well as litter exchanged between sites, will also be analyzed by three-way ANOVA, with sites, dates and litter types (Provenance) as main factors. Interaction between sites and litter types would here be of interest, because it would indicate differential decay of a given litter type with and without ELF influence, and would lead to further analysis of individual treatment cells.

VII. GENERAL CONCLUSIONS

In the forest systems we are monitoring for potential ELF effects, there are essentially three levels of investigation which can be pursued:

a) at the level of populations, quantifying numerical fluctuations within abundant species, and including effects of environmental variables as modifiers of seasonal and yearly patterns;

b) at the level of communities, documenting changes in abundance of component species;

c) at the level of the entire system, the functioning of which reflects all the complex interactions and feedback links between above- and below-ground subsystems.

Within the scope of this project, we cannot document all of the dynamic interchanges between major system components (microflora, fauna, and higher plants), necessitating selection of potential indicator taxa and processes. After several years of Test/Control monitoring, certain target parameters have indeed emerged at each of the above three levels. Some parameters have proven statistically tight, either in terms of Test/Control comparison or in terms of year-to-year variability within Test alone. Others seem less useful for project goals; yet, these may become important explanatory tools for long-term trends or, simply, provide evidence of the range of variation which exists independently of potential electromagnetic effects.

Based on data at hand, we propose that the main objectives we are now focusing on are of continued value to project goals.

a) At the level of single populations, several potential indicator species which are abundant enough for between-site analysis have emerged. They

include representatives of the most abundant taxonomic groups (Collembola, Acari, Carabidae, Lumbricidae) as well as of major functional or trophic categories (e.g., predatory and fungivore/detritivore arthropods, litter-feeding and soil-ingesting earthworms). The specific population attributes which can be quantified, however, are determined by the physiological and behavioral characteristics of the species themselves.

For soil/litter arthropods, abundance estimates have been highly variable. Although recently begun non-parametric analyses indicate synchronous seasonal fluctuations in Test and Control, density estimates may be somewhat insensitive. Nevertheless, they furnish important background data for long-term ecological interpretation. Patterns of reproduction and development, on the other hand (e.g., in Asca aphidioides), have not differed between sites and provide a detailed data base for future comparisons.

For some species, population densities cannot be assessed by non-destructive means (carabid beetles, velvet mites). In their case, seasonal and diel activity patterns, as they vary in response to temperature, provide behavioral indicators which are independent of absolute population size and which have shown quantifiable synchronicity in Test and Control. Since "activity" represents movement in search of food, mates, etc... it can be viewed as requisite for a species' success. Timing and degree of activity could thus influence reproduction, and we have begun to quantify seasonal changes in ovarian development and fecundity for carabids shared between sites. The data base is not yet complete, but these reproductive parameters promise sensitive analysis of potential ELF effects.

In the case of earthworms, we have been able to develop an extensive data base including spatial distribution, reproduction and recruitment in

relation to environmental factors. Continued sampling of Test lumbricids is obviously indicated. Seasonal and yearly fluctuations in major population parameters, as affected by climatic events during pre-ELF years, form the background against which potential disturbance can be measured. The value of concurrently monitoring earthworm populations in Control is somewhat limited by discrepant species composition and differing relative dominance of shared species. However, population phenology of the shared epigeic Dendrobaena octaedra and of the behaviorally similar endogeics Aporrectodea turgida - A. tuberculata pair provide detailed tools for direct site comparison.

b) At the level of communities, species composition and diversity have been target parameters. Litter and soil Collembola, earthworms, and the surface-active component of collembolan and carabid faunas have so far been examined. Based on yearly total numbers of any given faunal group, diversity indices have not been constant between sites or years. Aspects of community structure other than diversity (i.e., richness and evenness) have not yet been explored, but the necessary data base is available for further manipulation. In any case, whether or not these community parameters prove sensitive to detection of disturbance, they provide background information useful for biological interpretation of events at the population or system levels.

c) At the system level, we have selected two main target parameters: litter inputs and litter decomposition. Production of leaf litter is a parameter which integrates above-ground primary production and the litter/soil subsystem (through feedback between tree growth and decomposition). Litter decomposition, in turn, is an integrative measure of system function, reflecting the combined effects of all biota instrumental in organic matter break-

down and nutrient release.

Both litter production and decomposition (in terms of mass loss over time) have proven relatively constant between sites and years during the pre-ELF period. These system-level parameters are thus valuable tools for detecting potential perturbation. In addition, because they are interactive with the biological characteristics of decomposer species, they provide a reciprocal check for certain faunal parameters under investigation (earthworm and arthropod density and activity). We believe that these varied lines of approach at several levels constitute a major strength of this project.

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APPENDIX A

Publications and manuscripts

Publications

- Walther, P.B. and R.M. Snider. 1984. Techniques for sampling earthworms and cocoons from leaf litter, humus and soil. *Pedobiologia* 27: 293-297
- Snider, R.M. and R.J. Snider. 1986. Evaluation of pit-trap transects with varied trap spacing in a northern Michigan forest. *Great Lakes Ent.* 19: 51-61
- Sferra, N. 1986. First record of Pterodontia flavipes (Diptera: Acroceridae) larvae (Diptera: Acroceridae) in the mites Podothrombium (Acari: Trombididae) and Abrolophus (Acari: Erythraeidae). *Ent. News* 97: 121-123
- Snider, R.J. and R.M. Snider. 1987. ELF ecological monitoring in Michigan. I. Description of sites for soil biological studies. *Pedobiologia* 30: 241-250
- Snider, R.J. and F.J. Calandrino. 1987. An annotated list and new species descriptions of Collembola found in the Project ELF study area of Michigan. *Great Lakes Ent.* 20: 1-19

Manuscripts submitted and in preparation

- ELF ecological monitoring in Michigan. II. The earthworm communities of Test and Control sites. (To be resubmitted April 1988).
- ELF ecological monitoring in Michigan. III. Phenology of Dendrobaena octaedra (Lumbricidae) in Test and Control sites.
- ELF ecological monitoring in Michigan. IV. Breeding periods and activity patterns of Carabidae in Test and Control sites.
- Phenology of Lumbricus rubellus and Aporrectodea spp. (Lumbricidae) in northern Michigan forests.
- Breakdown of sun and shade leaves of sugar maple in two deciduous forest sites in Michigan.

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ELF Communications System Ecological Monitoring Program

BIOLOGICAL STUDIES ON POLLINATING INSECTS: MEGACHILID BEES

Annual Report 1987

Karen Strickler
J. Mark Scriber
Department of Entomology

FRONTISPAGE

Subcontractor: Michigan State University
East Lansing, Michigan 48824

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Department of Entomology

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TABLE OF CONTENTS

LIST OF TABLES	vi
LIST OF FIGURES	viii
I ABSTRACT	1
II INTRODUCTION	3
Project Rationale and Overall Objectives.	3
Nesting Biology of Megachilid Bees	4
Hypotheses Tested	5
Hypotheses involving nest architecture	6
Hypotheses Involving Nest Activity	8
Hypotheses Involving Emergence	9
III METHODS AND TYPES OF DATA COLLECTED	11
Trap Nesting Methodology	11
Nest Architecture Measurements	13
Emergence Data	13
Leaf Counts	14
Nest Activity	14
Statistical Methods	16
IV NEST ARCHITECTURE RESULTS	19
Bee Abundance	19
Hypothesis 1	19
<u>M. relativa</u>	20
<u>M. inermis</u>	22
Number of cells per nest	23
Hypothesis 2	24
Hypothesis 3	25
Hypothesis 4	25
V NEST ACTIVITY RESULTS	27
Sample sizes	27
Hypothesis 5	28
VI EMERGENCE RESULTS	29
Hypothesis 6	29
VII SUMMARY	31
VIII REFERENCES	35
IX LIST OF ACRONYMS	37

LIST OF TABLES

TABLE 1: Bore size categories, and diameter of drill bits associated with each category.	38
TABLE 2: Number of nests of <u>M. relativa</u> and <u>M. inermis</u> at each site for which we have data on cell lengths.	39
TABLE 3: GLM of all cells from 1985 and 1986 <u>M. relativa</u> nests; Cell lengths.	40
TABLE 4: GLM of all cells from 1985 and 1986 <u>M. relativa</u> nests; Cell volumes.	41
TABLE 5: GLM of cells in bore size 4 nests, 1985 and 1986 <u>M. relativa</u> nests; Cell lengths.	42
TABLE 6: GLM of cells in bore size 4 nests, 1985 and 1986 <u>M. relativa</u> nests; Cell volumes.	43
TABLE 7: GLM, including date of nest initiation, 1985 and 1986 <u>M. relativa</u> nests (except 1986 F1); Cell lengths.	44
TABLE 8: GLM, including date of nest initiation, 1985 and 1986 <u>M. relativa</u> nests (except 1986 F1); Cell volumes.	45
TABLE 9: GLM of cells with leaf counts, 1986 <u>M. relativa</u> nests; Cell lengths.	46
TABLE 10: GLM of cells with leaf counts, 1986 <u>M. relativa</u> nests; Cell volumes.	47
TABLE 11: GLM of cells for which offspring's sex is known; 1985 and 1986 <u>M. relativa</u> nests; Cell lengths.	48
TABLE 12: GLM of cells for which offspring's sex is known; 1985 and 1986 <u>M. relativa</u> nests; Cell volumes.	49
TABLE 13: Differences between observers in mean cell lengths and cell volumes (<u>M. relativa</u> , bore size 4 only).	50
TABLE 14: Mean cell length by cell order in the nest.	51
TABLE 15: GLM of all cells, 1985 and 1986 <u>M. inermis</u> nests; Cell lengths.	52
TABLE 16: GLM of all cells, 1985 and 1986 <u>M. inermis</u> nests; Cell volumes.	53
TABLE 17: GLM of cells in bore size 7 nests, 1985 and 1986 <u>M. inermis</u> nests; Cell lengths.	54

TABLE 18: GLM of cells in bore size 7 nests, 1985 and 1986 <u>M. inermis</u> nests; Cell volumes.	55
TABLE 19: GLM, including date of nest initiation, 1985 and 1986 <u>M. inermis</u> nests (except 1986 F1); Cell lengths.	56
TABLE 20: GLM, including date of nest initiation, 1985 and 1986 <u>M. inermis</u> nests (except 1986 F1); Cell volumes.	57
TABLE 21: GLM of cells for which offspring's sex is known; 1985 and 1986 <u>M. inermis</u> nests; Cell lengths.	58
TABLE 22: GLM of cells for which offspring's sex is known; 1985 and 1986 <u>M. inermis</u> nests; Cell volumes.	59
TABLE 23: Categorical modeling of number of cells per complete nest of <u>Megachile relativa</u>	60
TABLE 24: Mean number of leaves lining cells, by cell order in the nest, and by bore size.	61
TABLE 25: Number of individual bees of <u>M. inermis</u> and number of LO trip durations timed by each observer at each site, 1983-1986.	62
TABLE 26: Number of individual bees of <u>M. inermis</u> and number of LO trip durations timed by each observer at each site, 1987.	63
TABLE 27: GLM of log transformed LO trip durations for <u>M. inermis</u> , 1987.	64
TABLE 28: GLM of log transformed LO trip durations for <u>M. inermis</u> , 1983 - 1986.	65

LIST OF FIGURES

Figure 1. Cut away view of a completed <u>Megachile</u> nest.	67
Figure 2. Arrangement of nests in block, 1983 - 1986.	68
Figure 3. Arrangement of nests in block, 1987.	68
Figure 4. Hutch, with one block of nests.	69
Figure 5. A single reproductive cell, indicating how cell lengths are measured.	70
Figure 6. Cumulative number of nests of <u>M. relativa</u> and <u>M.</u> <u>inermis</u> at each site, 1985-1987.	71
Figure 7. Distribution of reproductive cells per nest for <u>M.</u> <u>relativa</u> at the F2 site.	72
Figure 8. Distribution of reproductive cells per nest for <u>M.</u> <u>inermis</u> at the F2 site.	73
Figure 9. Distribution of nest plug lengths for <u>M. inermis</u>	74

I ABSTRACT

High voltage transmission lines and magnetic fields have been shown to affect honeybee reproduction, survival, orientation, and nest structure. ELF EM fields could have similar effects on native megachilid bees.

Two species in the genus Megachile have been most abundant in artificial nests at experimental and control sites in Dickinson and Iron Counties. Data on their nest architecture, nest activity, and emergence/mortality have been collected since 1983. Five hypotheses concerning the possible effects of ELF EM fields are considered using these data. Thus far, we have not detected significant differences between experimental and control areas in cell lengths, cell volumes, number of cells per nest, or time to collect a leaf to cap a cell. Furthermore, there are no significant differences in the effect of year on experimental and control areas in these factors. Sample sizes similar to those obtained in 1987 should be sufficient to detect reasonable differences between experimental and control areas in cell lengths, cell volumes, and time to collect a leaf cap. Only large changes in cells per nest are detectible for the smaller Megachile species. Further analyses of the data are continuing.

II INTRODUCTION

Project Rationale and Overall Objectives.

Effects of high voltage transmission lines and fluctuations in the earth's magnetic field have been reported to affect honeybees (Greenberg et al. 1981; Gould 1980). In addition, honeybees have been shown to have an organ in the abdomen that could be used to detect the earth's magnetic field and thus could be used as a compass in orientation (Gould et al. 1978). Because such effects of electric and magnetic fields have been demonstrated, it is possible that ELF EM fields may alter a bee's ability to orient or may otherwise affect its behavior.

Honeybees, however, are rare in the state forest where the Michigan ELF antenna is located, and are unable to overwinter in the harsh climate of Michigan's Upper Peninsula (Fischer, 1983 Annual Report). Therefore, native bees are a better choice for ecological studies of the resident bee fauna. Native bees are particularly important in ecological communities such as those in the vicinity of the ELF antenna because they are pollinators of flowering plants, and are therefore important to the reproductive success of these plants.

With the exception of bumblebees and some halictids, native bees are solitary, meaning that each female constructs and provisions her own nest rather than having a special queen caste responsible for reproduction. Solitary bees have several advantages for ecological studies. As "mass provisioners", they create a discrete cell for each offspring, and fill it with a provision mass of pollen and nectar prior to laying the egg. The bee does not add more provisions after the egg is laid. A series of such cells, each with a provision mass and egg, are created in succession by each female. The provisions that go into each cell are a direct measure of parental investment in an offspring (Strickler, 1979). The size of the adult bee that emerges from each cell is correlated with the amount of provisions provided it, and with the size of the cell in which the larva develops (Krombein 1967; Klostermeyer et al. 1973; Torchio and Tepedino 1980). However, there is a tradeoff between the investment per offspring and the rate at which offspring are produced. The more the bee invests per offspring (ie, the larger the offspring), the fewer offspring she will produce. If bees are disoriented, agitated, or slower at foraging, they may invest less per offspring, produce fewer offspring per unit time, or both. Solitary bees are unusual in having this direct relationship between parental investment per

offspring, adult size, and reproductive output.

The nesting biology of some species of solitary bees in the family Megachilidae is especially easy to study because they accept artificial nests in the field. These bees typically nest in abandoned beetle bores in dead logs. "Trap nests" of drilled blocks of wood are also used by bees as nest sites. Such artificial nests can be placed in habitats where bees are expected to nest, in order to increase the sample of nests available for study, and to standardize such characteristics of the nest as bore depth and diameter (Krombein, 1967). Trap nests are used in the management of the leafcutter bee, Megachile rotundata, for pollination of alfalfa (Hobbs, 1972). Thus there is an extensive (though unreviewed) literature on megachilid biology.

Research on the effects of high tension wires and magnetic fields on honeybees suggests working hypotheses on which to base our initial analyses of native bee nesting biology. Of possible relevance to megachilid behavior are an alleged greater tendency for dispersal, and greater levels of activity (Wellenstein, 1973), as well as reduced reproductive output, lower overwintering survival, and modifications of nest structure (Greenberg et al., 1981) when colonies were exposed to electromagnetic fields from high voltage transmission lines. In addition, disorientation due to fluctuations in ELF magnetic fields is possible if megachilids share the honeybee's ability to detect magnetic fields. (Gould et al., 1978, 1980; Gould 1980; Tomlinson et al. 1981).

Nesting Biology of Megachilid Bees

A decision to restrict our study to two species of leafcutter bees, Megachile relativa and M. inermis, was made in the fall of 1986 (1986 Annual Report). M. inermis and M. relativa have similar nest architecture in that both line their cells with pieces of cut leaves. However, the two species differ in size, and may therefore partition their time and the space in their nests differently.

The general structure of the nests of the two species is depicted in Fig. 1. The bee may leave some space at the base of the nest (the basal space) unoccupied by cells for offspring. She may then cut and bring to the nest a few round pieces of leaf which are added one at a time to form the base of the first cell. Next she cuts and brings to the nest several elongate pieces of leaf in succession. These are used to line a tube- or cup-shaped cell that is slightly longer than her body. Next she makes a series of pollen and nectar foraging trips to fill the cell with the discrete provision mass that will be the larva's food supply. When

provisioning is complete, the female lays an egg. Fertilized eggs become females while unfertilized eggs become males. The female has voluntary control over the sex of the egg that she lays (Klostermeyer and Gerber, 1970). After laying the egg, she cuts more round leaves to cap the cell, sometimes adding chewed leaves, sand, pebbles, or bits of wood to separate the cells. Next she cuts more elongate leaves for the second cell, and repeats the process. Thus a linear series of cells is constructed in the nest bore. Typically, the cells at the base of the nest are more likely to contain females and the cells near the entrance are more likely to contain males (Krombein, 1967). Since females are usually larger than males in these bees, cells at the base of the nest tend to be larger than cells at the entrance. When she has completed the last cell that she is going to put in the nest, she constructs a series of plugs of round leaves, chewed leaves, and possibly other material. M. relativa frequently includes empty "vestibular" spaces between segments of plug. M. inermis and some M. relativa create one long mass of plug material after completing the reproductive cells. In nests of both species there may also be space between the outermost plug and the opening of the nest, called an "indentation".

Each female may construct several such nests over her life time. Some nests are abandoned before they are finished because the bee has died, or for other unknown reasons.

Inside each cell the egg hatches, and the young larva feeds on the provisions prepared by its mother. Both Megachile species in our study are univoltine in Northern Michigan, and both overwinter as prepupae. Pupation occurs in Spring, and adults emerge soon after, in mid-June at our study sites. A variety of parasites may emerge from the cell instead of the original bee. Oviposition of parasite eggs usually occurs while the cell is being provisioned, when the mother bee is out of the nest on a pollen foraging trip, or on a round-leaf foraging trip just after laying her egg.

Hypotheses Tested

During the first four years of the project, 1983-1986, data on nest architecture, nest orientation, emergence/mortality and nest activity were collected. Based on these data, six tentative hypotheses concerning the effects of ELF EM fields on Megachile behavior were specified in the 1986 Annual Report. The initial hypotheses were modified in last year's report based on our ability to gather sufficient sample sizes to detect differences between experimental and control areas. The modified hypotheses are expressed in the following sections as null hypotheses, i.e., hypotheses of no difference between experimental and control areas, that we

will try to disprove statistically. The "Rationale" sections explain the possible effects of ELF EM fields that may cause a rejection of the null hypothesis.

Hypotheses involving nest architecture:

Hypothesis 1: The average size (length and volume) of cells for each offspring, and/or the average number of cells produced per nest is unchanged by exposure to ELF electromagnetic fields.

Rationale

Honeybee reproductive output decreased on exposure to high voltage transmission lines (Greenberg, et al., 1981). ELF EM fields may have a similar effect on megachilids. The ELF electromagnetic fields may affect cell size and nest architecture in various ways. For example, if bees are disoriented by the fields, they may gather resources (leaves, pollen) more slowly when exposed to the fields than when not exposed. As a result, they may produce new cells at a slower rate, or they may produce smaller cells.

Previous studies have found that the weight of offspring of the generalist megachilid, Osmia lignaria, is lower if their cells were produced late in the season rather than early in the season (Torchio and Tepedino, 1980). This species also showed an increase in the proportion of male offspring (the smaller sex) produced late in the season. A reduction in offspring size late in the season is thought to be related to reduced foraging rates due to aging of the bee (Torchio and Tepedino, 1980, Tepedino and Torchio, 1982). Similarly, ELF EM fields may slow the foraging of M. relativa and M. inermis, resulting in smaller bees produced in smaller cells. A size reduction could affect cells with offspring of both sexes, or it could reflect the production of a greater proportion of male offspring, for species with smaller males than females. An additional complication is that female sizes decrease more than male sizes late in the season (Torchio and Tepedino, personal communication). Thus we might expect female cells to be affected more than male cells by stresses from ELF EM fields.

In contrast to the generalist megachilids, the pollen specialist Hoplitis anthocopoides did not show a reduction in offspring weight late in the season, in spite of reduced foraging rates (Strickler, 1982). Rather, it was hypothesized that slower foraging rates led to fewer offspring per nest late in the season as compared with early in the season for this species. Similarly, M. relativa and M. inermis may produce fewer cells per nest in response to slow foraging rates due to ELF EM fields.

In testing hypothesis 1 we are interested in determining whether there are differences between experimental and control sites in cell lengths, cell volumes, and number of cells per nest. Ideally, we hope to find no differences between experimental and control sites, and between years, prior to the 1987 season when the ELF antenna was operational at low power. Then, if significant differences between experimental and control sites appear in the years after the antenna is turned on, we can attribute these differences to the effect of ELF EM fields.

Hypothesis 2. Bees exposed to ELF EM fields, and bees not exposed, will make nest plugs of the same thickness and will devote the same proportion of nest space to reproduction.

Rationale

Honeybees increased the amount of propolis at their nest entrances under high voltage transmission lines, presumably in response to stress connected with electric fields at the nest entrance (Greenberg et al, 1981). This suggests the possibility that megachilid bees will respond to disturbance from ELF EM fields by increasing the amount of nest lining material in the bores. This may be reflected in larger cells (tested in hypothesis 1) and/or increased nest plug length. More generally, there could be an increase in the nest space that does not include cells for offspring (ie. basal and vestibular spaces, nest plugs and indentations).

Hypothesis 3. The number of leaves used to line a cell is unchanged when bees are exposed to ELF EM fields.

Rationale

Bees may pad a cell with extra leaves as a result of stress due to electromagnetic fields (see hypothesis 2). Originally we had planned to test this hypothesis using nest activity data, by counting the number of elongate leaf (LR) collecting trips taken by a nesting bee. However, in the 1986 Annual Report we concluded that the time available to test this hypothesis by watching bee activity would not yield sufficiently large sample sizes to detect differences between experimental and control areas. Instead, we proposed at that time to determine the number of LR leaves used to line a cell by taking the cell apart after bee emergence.

Hypothesis 4. The relative acceptability of nests oriented in a NS direction vs. nests oriented in an EW direction does not change when bees are exposed to ELF EM fields.

Rationale

Honeybees may use the earth's magnetic field under special circumstances to orient their comb (reviewed in Gould, 1980). The fluctuating ELF magnetic fields could disturb any biases that megachilids normally have for nest orientation, or could cause greater acceptance of nests oriented in certain directions in order to reduce disturbance by the fields.

Hypotheses Involving Nest Activity

Hypothesis 5. The duration of round leaf (LO) foraging trips remains the same when bees are exposed to ELF EM fields.

Rationale

Honeybee activity allegedly increased under high voltage electromagnetic fields (Wellenstein, 1973). If ELF EM fields cause a similar effect on megachilid bees, the time of leaf- and pollen-foraging trips might decrease. Alternatively, bees could become disoriented or agitated in the field, so that their foraging trips increase in duration.

Leaf-foraging trips for M. inermis and M. relativa are easy to recognize behaviors, usually lasting less than a minute in duration. Many of these trips are taken in succession, so within and between bee variability can be analyzed, and a potentially large sample of leaf collecting trips can be timed. In the 1986 Annual Report we demonstrated that the collection of LO leaves was the most consistent behavior of the leaf-cutting bees under study. We argued that this is probably because it is adaptive to close the cell as quickly as possible after the egg is laid so parasites don't get into the cell and destroy the offspring. Thus, this year's analysis focuses on LO trip durations.

Hypotheses Involving Emergence:

Hypothesis 6. Overwintering survival of megachilid bees is unchanged by exposure to ELF EM fields.

Rationale

High voltage transmission wires lower the overwintering survival of honeybee colonies (Greenberg et al., 1981). We would like to test for a similar effect in megachilid bees. Ideally, we would like to compare control and experimental sites in the proportion of cells that suffer various sources of mortality (parasitism, death of eggs, larvae, pupae, and adults) and the proportion that molt to the adult stage and emerge. This involves examining emergence data for the nests.

III METHODS AND TYPES OF DATA COLLECTED

Nest architecture and nest orientation are obtained by placing trap nests in the environment, and allowing bees to construct nests in their choice of traps during the summer. The following spring, various parameters of their nest architecture are measured. Bee and parasite emergence and larval and pupal mortality are recorded at the same time. Nest activity data are gathered during the summer season while the bees are constructing their nests.

Some changes in protocol were recommended in the 1986 Annual Report that were implemented during the 1987 season. The methods discussed below will compare, where appropriate, 1987 and pre-1987 methodology. Where no such comparisons have been made, no significant change in protocol has been made.

Trap Nesting Methodology

Trap nests consisted of elongate white pine pieces 19x19x153 mm. drilled lengthwise to a depth of either 142mm (smaller diameters) or 107mm (largest diameter pre-1987, and half of the 1987 nests). Prior to 1987, six different bore "sizes" corresponding to the diameters of six different drill bits, were used (Table 1). (Note that the bore diameters are not associated with consecutive bore sizes). Twelve nests, two of each bore diameter, were bound together with plastic strapping in to a "block", so that one of each bore size faced each direction, and no two bore entrances were adjoining (Fig. 2). In 1987 only bore sizes 4 and 7 were used (5.5mm and 11.0mm) because these sizes were accepted most often by the two Megachile species under study in previous years. Again, 12 nests were bound together into a block. Two large and four small bore sizes were arranged randomly in each direction (Fig. 3). We did not realize that the 1987 arrangement of nests differed from previous years until blocks for 1987 had already been prepared. However, we do not believe that this change in nest arrangement affected the bee's behavior.

"Hutches" consisting of a wooden frame with four shelves and a roof were used to hold the blocks of trap nests (Fig. 4). Four blocks of nests were placed randomly on each shelf, making a total of 192 nests present at any one time. The hutch was open on both sides, so half of the nests opened in each direction. The shelves were roughly 0.1, 0.4, 0.8, and 1.1 meters from the ground.

Four study sites were selected in 1984 for placement of hutches. Two are experimental sites along the ELF antenna:

Ford 1 and Ford 2 (F1 and F2), and two are control sites: Camp 5 and County Line (C5 and CL). Further information on the study sites can be found in the 1985 annual report. Three sets of two hutches, making a total of six hutches were placed at each of the four study sites. In each set of two hutches, one hutch was oriented in a north-south direction so that its nests open to the east or west, and one hutch was oriented in an east-west direction so that its nests open to the north or south. The two hutches in each set were placed close together in edge habitats between open areas where there are abundant flowering plants, and woods where natural nest constructing materials are available.

When a nest was occupied by a megachilid bee, it was given a number that included site, hutch direction, bore direction and shelf height. This number was written on the side of the nest. Position on the shelf and in the block of nests was not recorded. In 1987, a computer data base was created to help us keep track of nest numbers and progress of the nesting bee.

Once a nest in progress was identified, the depth of bore not yet filled in nest construction was recorded daily (pre-1987) or every 2-7 days (1987). This information, coupled with nest architecture measurements taken the following spring, allowed us to determine which cell the bee was constructing on the day the nest was first located. Assuming that the bee takes approximately one day to complete a cell, we estimated the dates on which the nest was begun and finished. When the nest was completed, it was removed from the block, and replaced with a nest of the same bore size.

Prior to 1987, completed nests were brought to Channing to overwinter, in order to avoid vandalism and marauding animals. In 1987, nests were left at the site that they were created, oriented in their original direction. Each nest was stored in a large centrifuge tube with cloth covering the opening. Tubes were placed in overwintering wooden boxes built to fit the hutch shelves. However, the 1987 nests were not stored overwinter on hutch shelves, but rather were elevated about a foot off of the ground and camouflaged with branches, bark, and leaves in order to avoid vandalism. The overwintering boxes were still in good condition at all of the sites when retrieved in May, 1988.

Low numbers of M. inermis nests at the CL site, especially in 1986, prompted us to transplant about 90 Cirsium spp. plants (a common pollen source at other sites) to the CL site in April, 1987 to try to increase the numbers of M. inermis that nested there.

Nest Architecture Measurements

After recording nest number and bore diameter, nests were split open lengthwise with a chisel. Total bore length, non-reproductive spaces (basal space, vestibular spaces, associated caps, nest plugs, and indentation) were measured with the cells intact. Each cell was then removed and measured from the base of the cell to the position of the outermost leaf in the cell cap (Fig. 5).

Since the nest number includes information on the site where the nest was created, measurements were not blind. We doubt that knowledge of the nest site affected our measurements. However, in response to reviewer comments, we will make blind measurements of the 1988 nests (1987 measurements had already been completed when the comments were received). We can do this by having one person record nest number on the reverse side of architecture data sheets, and open the nest. Another person will record measurements without seeing nest number until after measurements are complete.

Cell caps are enclosed in leaf linings, and are destroyed when the bee emerges, so we have abandoned the idea of measuring cell caps, as proposed in the 1986 Annual Report.

When more than one person measured nests, an attempt was made to divide the nests equally by site and date of nest initiation among all measurers. Thus individual biases in measurement would be distributed evenly between sites and dates. In addition, 20 M. relativa cells were re-measured to determine within- and between-individual measurement error. Each cell was measured three times by each of the four individuals measuring nests. The order in which each individual measured the cells was also recorded to determine whether increased handling of the same cell affects the length measured.

Estimates of cell volumes were calculated using cell length and bore diameter measurements, and assuming that the cells were cylindrical.

Emergence Data

Nests created in 1985 were checked daily in the spring of 1986 for bees that had emerged from the nest and were in the tubes. After measurement of 1986 nests in Spring, 1987, cells from which nothing had yet emerged were placed in individual plastic culture tubes or rearing dishes, and labeled with nest and cell identification numbers. Tubes

were kept indoors at room temperature (approx. 68°F) until emergence. In both years, date of emergence, species, and sex of offspring were recorded.

In 1987, two or three bees from each 1986 M. relativa nest were saved for dry weight measurements and for confirmation of species identification. Since these individuals were collected within hours of emergence without being released, their crops were empty. Thus much of the variability in weights that would be expected from a sample of field collected bees was eliminated. Weights were obtained by drying in a desiccator over P_2O_5 to constant weight (two weights within 0.5mg). The lowest weight was used in analyses.

Bees were identified by G. Dahlem and K. Strickler based on Mitchell (1962), and by comparison with reference specimens provided by T. Griswold, ARS Bee Laboratory, Utah State University, Logan Utah.

The remaining adult bees were released at the sites where their nest had been constructed the previous summer. Parasites were collected and not released.

Cells that showed no signs of emergence were opened in August, (1986 nests) or when the nest was measured (1985 nests). Contents were recorded to indicate at what stage death had occurred.

Leaf Counts

The number of elongate leaves that were used to construct a cell was determined for all 1985 M. inermis cells and 1986 M. inermis and M. relativa cells that were still in good condition once emergence was complete. Leaves lining M. inermis cells overlapped, but were easy to tease apart and count. Leaves lining M. relativa cells were smaller, and were "glued" together so that a microscope was often needed to determine where one leaf ended and the other began. When in doubt, leaf counts for M. relativa cells were not recorded.

Nest Activity

One or more observers have gathered data on behavior of individual bees at the nest every year since 1983. In the 1986 Annual Report, we decided to focus on the collection of round pieces of leaf (LO trips) used in capping a cell. Last year's analysis suggested that this was the most consistent of the three main behaviors in nest construction (collection

of pollen, elongate leaves, and round leaves). Thus the duration of these trips could be normalized for statistical analysis.

Prior to 1987 each observer watched a single bee for several days in succession, until the nest was complete. This protocol generated a great deal of information on the variability in behavior within a bee, but less information on between-bee variability. In 1987 we maximized the number of bees timed per day, rather than timing one bee for long periods of time. Observers became adept at locating a bee that was about to lay her egg, and were able to focus on timing the first few LO trips that the bee made after laying her egg. Generally, we tried to time 5 such trips in succession before searching for another bee that was about to collect LO leaves. Occasionally the bee would complete a cap in fewer than 5 timings. The observer sometimes would time more than 5 LO trips if no other bees were active. Number of trips timed for a bee on a given day ranged between 1 and 18. Occasionally the observer missed recording the time of the first few trips. Unfortunately, we did not try to record the number of LO trips that the bee had already made before we began timing. Our analysis suggests that this number is important (see results, below), so we plan to record it during the 1988 field season.

In 1987, four observers were rotated between sites every 3 to 4 days, so that biases between observers would be distributed evenly between sites and dates. On a given day, two observers visited a control site and two an experimental site. The P.I. also timed bees on a few occasions, especially early in the season when the observers were being trained.

Prior to 1987, the duration of LO trips was determined by using a watch to record the hour, minute, and second that the bee left the nest and returned to the nest. During 1987, we used portable Tandy 102 computers that were programmed as event recorders. When the program was activated, the observer was prompted for information on the nest number and site, and some weather data (see below). The program automatically numbered the observed activities in sequence. Hitting the space bar recorded the time to the nearest second at which the bee left the nest or returned to the nest. A single letter code was used to indicate what cargo (e.g., LOs), if any, the bee brought back to the nest. These data were down-loaded to a Zenith personal computer at our field headquarters, and later transferred to the VAX 11/730 computer (VAX/VMS operating system) in the Department of Entomology at MSU. Durations of each trip were calculated by subtracting the time when the bee left the nest from the time when the bee returned.

Because behavior of insects is often affected by such environmental factors as temperature and wind speed, foraging trip durations could be correlated with weather conditions. In previous years, air temperature, relative humidity, solar radiation, rainfall, barometric pressure, wind direction, and wind speed were monitored automatically with Model TI-5X instrumentation modules at one experimental (F1) and one control (CL) site. The instrumentation did not always function properly. In 1987 we did not have time to set up the automatic weather equipment until the beginning of August. Then we principally wanted to determine which data pods were functional and which were not. Soon after setting it up, one of our batteries was stolen. We have not had time to evaluate the availability of weather data from these automatic systems, or to attempt appropriate correlations.

Some weather data were recorded in the event recorders as each bee was timed. This included sun conditions (sunny, partly cloudy, cloudy, rain), temperature in the shade on the same shelf as the bee's nest, shading of the block in which the bee's nest was found, relative humidity calculated with a sling psychrometer, average wind speed and speed of wind gusts measured with a Dwyer Portable Wind Meter (hand held). Although our measurements of solar radiation, relative humidity, and wind speed may be crude, they are better than nothing (as we had in the past for C5 and F2), and may give a better indication of conditions around the nest than did the EPROM equipment, which was not always near the appropriate hutch.

Statistical Methods

A Shapiro-Wilk statistic for $N < 51$ and a Kolmogorov statistic for $N \geq 51$ in the Univariate procedure of SAS (Version 5) were used to test for normality of LO trip durations, cell lengths, cell volumes and leaf counts. The significance level used in these tests was 0.05. A log transformation was used for the LO trip durations.

The General Linear Models (GLM) procedure on SAS was used to analyze sources of variability in cell lengths, cell volumes, and LO trip durations. In all analyses, experimental vs. control areas (Exp) were treated as a random class variable. Sites nested in experimental and control areas (Sites[exp]), observers or measurers (doneby), and sex of offspring were treated as fixed class variables. Complete vs. incomplete nests were a random class variable in the analysis of cell length and cell volume. Cell order, number of cells per nest, nest diameter, number of leaves per cell, and date of nest initiation were covariates in the analysis of cell lengths and volumes. Rank order of the trip, time of

day, and date of the trip were covariates in the analysis of LO trip durations. Time was also tested as a second order covariate in this analysis. Significance would indicate that LO durations are faster (or slower) during the middle of the day, as might be the case if LO durations are correlated with temperature. All other variables were fixed in the analysis.

Cell length data for both 1985 and 1986 were analyzed simultaneously, so we tested for significance of the interaction between year and Exp. The same interaction term was included in the cell volume GLM model. If significant, this interaction term indicates that one area has shown a greater change between years than the other. Ideally this interaction term will not be significant before the antenna is operational. If the Exp main effect is significant but not the Exp*year interaction, then we know that there are intrinsic differences between experimental and control areas that have nothing to do with the antenna. If the year main effect is significant but not the Exp*year interaction, then we know that there are differences between years that have affected both experimental and control areas equally, as would be the case for climatic changes between years. If the Exp*year interaction is not significant before the antenna is operational, but it becomes significant after the antenna is operational, the antenna is a likely cause of the difference. If the interaction term is significant before the antenna is operational, then the problem of detecting differences between experimental and control areas will be much more complex.

Factors such as nest diameter, date of nest initiation, and offspring's sex are included in the model because if they contribute to variance in cell lengths and/or volumes now, then changes in these factors due to ELF EM fields are possible. Such changes will be the underlying cause of differences between experimental and control areas due to ELF EM fields, if such differences are found. For example, if sex of offspring contributes significantly to the variance in cell lengths before the antenna is operational, then cell lengths could decrease after the antenna is operational because a higher proportion of male offspring are produced.

Type IV mean squares were calculated in all GLM analyses. The mean square of site(exp) was the error term in hierarchical tests of significance of Exp and of the Exp*year interaction. The overall model error was used to test the significance of all other variables.

Minimum detectible differences between experimental and control areas were tested with a modification of Cochran and Cox's (1975) formula (Zar, 1984 p.135). Conservative sample size estimates were based on numbers actually collected in

1987 for the two control sites combined or the two experimental sites combined, whichever was smallest. The value of population variance s^2 , used in calculating minimum detectable differences, was the site[exp] mean square because this mean square value is used as the error term for testing Exp and Exp*year (Zar, 1984 p.260). Degrees of freedom used was 2, the degrees of freedom associated with the mean square. Values of α and the power of the test (1- β) were 0.05 and 0.75 respectively.

The Categorical Data Modeling (CATMOD) procedure on SAS was used to compare distributions of cells per nest from experimental and control areas. This statistical program fits linear models to functions of response frequencies for discrete data; ie., it is an extension of the GLM procedure for continuous data that was used in the analyses of cell lengths and volumes. The program uses a Wald statistic (which approximates a chi-square distribution for large sample sizes) to test hypotheses about linear combinations of the parameters in the model. As with the GLM tests previously described, we tested for significance of experimental vs. control areas (Exp), sites nested in Exp areas (Site [exp]), years, and the interaction between Exp and years (Exp*year). The level of significance of all tests was 0.05.

No simple tests are available to calculate the minimum detectible difference in cells per nest between experimental and control areas from a CATMOD analysis. Therefore, we used a "jackknife" technique to estimate minimum detectible differences. This involved randomly assigning the original data values to either experimental or control areas, in the same proportion as in the original data. Thus, any initial differences that exist between sites are eliminated. Each value that was assigned to the experimental area was then reduced by a stochastically generated number of cells per nest. The amount of the reduction was calculated with a standard normal variable whose mean was the desired average reduction of cells (one, two, three, etc.), and whose variance was one. 100 different realizations (modifications of the original data set) were made for each average cell reduction. Each realization was tested with the CATMOD procedure used on the original data. For a given average cell reduction, the number of realizations that showed significance of the Exp variable is an estimate of the power of the test. The minimum detectible difference was the average number of cells per nest by which the original data had to be reduced in order to detect a significant Exp variable in at least 75 of the 100 realizations of the test. This corresponds to a power of at least 75 for the test.

IV NEST ARCHITECTURE RESULTS

Bee Abundance

Table 2 summarizes the number of nests of the two species for which we have data on cell lengths, and an estimate of the number of complete nests created in 1987. Some 1985 M. inermis nests were not included in our measurements because they were used by Dr. Fischer in experiments on diapause. The 1983 nest architecture data have not yet been incorporated into our analysis because they are still being edited to make them comparable to the 1985-1986 data. 1987 nests will be measured in the spring of 1988.

Cumulative numbers of nests constructed each week at the four sites each year are presented in Figure 6. Final nest numbers are underestimates, especially for 1985 M. inermis (see above). There are differences between sites and years in dates of first and last nest construction, and in rates of nest construction through the season. M. inermis always began nesting after M. relativa, but the former species remained active longer.

Of the 90 Cirsium transplanted to the CL site, about one fourth of them survived and bloomed. This was probably not enough additional resource to attract bees to the area. We hope that our efforts were not entirely in vain, since thistle seeds produced by the transplants may increase the Cirsium population in future years.

Hypothesis 1: The average size (length and volume) of cells for each offspring, and/or the average number of cells produced per nest is unchanged by exposure to ELF electromagnetic fields.

1985 nests were measured in November and December, 1986 (M. relativa) and August, 1987 (M. inermis) after bee emergence. Most 1986 M. relativa nests were measured before emergence in 1987, so that we would know with certainty the species and sex of the occupant of each cell. The 1986 M. inermis began to emerge in spring, 1987 before we had finished measuring M. relativa nests, so most of the (M. inermis) nests were measured after bee emergence. During the 1988 field season we will begin measuring M. relativa nests early enough that we can start on M. inermis before they emerge. This was difficult in 1987 because we had to spend some time in the spring setting up a new laboratory.

M. relativa

The GLM procedure eliminates from the analysis records that are incomplete for any of the variables in the model. A large number of cell records did not include date of nest initiation, number of leaves per cell, and sex of offspring for this species. Therefore the GLM test was repeated several times with different subsets of the data. First, we tested cell lengths and volumes with the maximum number of cells (Tables 3,4), by not including the above factors in the model. Second, we tested only cells from nests of bore size 4 (Tables 5,6), since these will be most comparable with nests from 1987 and future years. The Site[exp] mean squares from these GLMs were used to estimate minimum detectable differences between control and experimental sites (see below). The third GLM test included date of nest initiation in the model (Tables 7,8). All 1986 F1 nests were excluded from this analysis because we could not find the 1986 daily nest check information, used to determine nest initiation date. Number of leaves per cell, only available for 1986 nests, were included in the fourth GLM test (Tables 9,10). Finally, the fifth GLM test included only the cells for which the sex of the M. relativa offspring was certain (Tables 11, 12).

Mean cell length was 10.9mm and mean cell volume was 0.29 cm³ for M. relativa. Variance in both factors is low, only 8-10% of the means (see CV in Tables 3-12). The models accounted for only 12-22% of the variance in cell lengths, but 73-88% of the variance in cell volumes (see r² in Tables 3-12).

In all of the GLM tests, Exp and Exp*year did not contribute significantly to variance in cell lengths or cell volumes. This is fortunate, because we can expect that significance of the Exp*Year interaction in the future, when the antenna is operational, will reflect significant effects of ELF EM fields on cell lengths and volumes.

Differences between measurers contributed significantly to variance in both cell lengths and volumes in all GLM tests (Table 3-13). Cell lengths and volumes also consistently decreased with cell order from nest base to cell entrance (Tables 3-12, 14). Cell length decreased slightly but significantly with nest diameter when all bore sizes are included in the sample tested, but not when only bore size 4 is tested (Table 5 vs. 3,7,9,11). However, cell volumes increase significantly with diameter in all tests (Tables 4,6,8,10,12). The high correlation between cell volume and nest diameter, but not between cell length and nest diameter, probably accounts for the greater proportion of the variance

explained in models of cell volume than in models of cell length.

When included in the model, date and offspring sex contributed significantly to cell lengths and volumes (Tables 9-12). Cell lengths and volumes decreased slightly but significantly with date of nest initiation. Cells with male offspring were significantly smaller on average (length=10.8mm, volume=0.29cm³, N=576) than cells with female offspring (11.4mm, 0.32cm³ N=86). When sex is included, the proportion of variance in cell lengths explained by the model is almost doubled in comparison to other tests (see r^2 , Table 11 vs. 3,5,7,9). Thus, separating cells so that sex can be associated with cell lengths proved to be an improvement in protocol that we plan to continue.

Number of leaves per cell contributed significantly to variance in cell volumes, but not to cell lengths. This probably reflects an increase in number of leaves with increasing nest diameter. Other factors in the model such as year, cells per nest, complete vs. incomplete nests, and sites were variable in whether or not they contributed significantly to variance in cell lengths and volumes.

Minimum Detectible Difference Between Experimental and Control Areas

Assuming a minimum of 20 nests per site, and an average of 4 cells per nest, we expect a minimum of 80 cells per site, and 160 cells per experimental and control areas. Using $s^2 = 15.69$ (Table 5, SS for Site[exp]/df), we calculate that we should be able to detect at least a 1.65mm difference (15% of the mean) in cell lengths between control and experimental areas. For the same sample size, we expect to detect a difference of 0.046 cm³ (16% of the mean) in cell volumes ($s^2 = 0.0120$, Table 6).

Offspring Weights

In the 1986 Annual Report we questioned the necessity to analyze the variance in cell volumes, because volumes are highly correlated with nest diameters. We suggested that the answer to this question depended on whether offspring weights correlate best with cell length or with cell volume. Dry weights of some M. relativa offspring from 1986 nests were measured in hopes of addressing this question. These weights have not yet been added to our SAS data set, so we have not had time to analyze the data.

After weighing, the bees were pinned and identified to confirm that they were, in fact, M. relativa rather than one

of several related bees of the same size. Of 108 males and 40 females identified as of this writing, all but two males were confirmed as M. relativa.

M. inermis

As with M. relativa, several GLM tests were applied to different subsets of the M. inermis data. The first GLM model (Tables 15,16) did not include date of nest initiation or sex of offspring. The second GLM (Tables 17,18) was the same as the first, except that only cells from nests of bore size 7 were tested. Date of nest initiation was included in the third analysis (Tables 19,20), but all F1 records had to be eliminated from the data set, as was the case for M. relativa. Finally, sex of offspring was included in the analysis (Tables 21,22), although only a small number of offspring could be associated with a specific cell. Number of leaves per cell was included in all of these models because values were determined for both 1985 and 1986 cells.

Mean cell length was 15.1 mm, and mean cell volume was 1.27 cm³ for M. inermis. As with M. relativa, variance in both factors was only 8-10%. The models of cell length account for 16-35% of the variance, while the models of cell volume account for 64-86% of the variance. As with M. relativa, the strong positive correlation between cell volume and nest diameter account for most of variance in cell volumes.

Year did not contribute significantly to variance in M. inermis cell lengths or volumes in any of the models tested. Exp and Exp*Year did not contribute to the variance in the models of cell length and most models of cell volume. When the maximum number of cells are tested (Table 16), cells from experimental sites had significantly greater volumes than cells from control sites. Furthermore, the difference between control and experimental sites was greater in 1985 than in 1986. Because the interaction is not significant for cell lengths, and when only nests of bore size 7 are tested, we suspect that the differences are related to a greater average diameter of nests used at experimental than at control areas. In future years only nests of bore size 7 will be used, so Table 18 will be the relevant comparison.

As with M. relativa, differences between measurers (doneby) consistently made a significant contribution to cell lengths and volumes. Cell lengths and volumes decreased significantly with cell order from base to nest entrance. Complete nests had slightly but significantly greater cell lengths and volumes than did incomplete nests. A negative correlation between cells per nest and cell volumes (and most tests of cell length) is probably due the fact that nests

with the largest bore diameter had the shortest bore lengths, and thus could hold fewer cells.

Offspring's sex contributed significantly to variance in both cell lengths and volumes (Tables 21,22), with male cells being smaller on average (length=15.1mm, volume=1.27cm³, N=299) than female cells (15.9mm, 1.42cm³, N=63). As with M. relativa, the proportion of variance in cell length explained by the model doubled when offspring sex was included. Cell lengths, but not cell volumes, decreased slightly but significantly with date of nest initiation (Tables 19,20). Other variables in the model such as sites and leaves per cell were significant in some of the models tested, but not in others. This inconsistency suggests that they are only minor contributors to variability in cell lengths and volumes for this species, if at all.

Minimum Detectable Difference Between Experimental and Control Areas

In 1986, a total of only 17 nests were constructed by bees at the control sites (Table 2). Assuming a minimum of 17 nests and 4 cells per nest, we expect a minimum of 68 cells each in the control and experimental areas. Using $s^2 = 1.563$ (Table 17, SS for Site[exp]/df), we calculate that we should be able to detect at least a 0.8mm difference (5% of the mean) in cell lengths between control and experimental areas. For the same sample size, we expect to detect a difference of 0.07 cm³ (5% of the mean) in cell volumes ($s^2 = 0.0109$, Table 18).

Number of cells per nest

Number of cells per nest ranged from 1 to 12 for M. relativa (eg., Fig. 7). In our CATMOD analysis we used four categories of cells per nest to insure that frequencies were greater than five per category for all sites and years. The categories were: nests with 1 or 2 cells, nests with 3 or 4 cells, nests with 5 or 6 cells, and nests with seven or more cells.

There were significant differences between sites in the distribution of number of cells per nest (Table 23). However, the distribution of cells per nest was not significantly different between experimental and control areas, nor between years. The interaction between years, and experimental and control areas was also not significant.

We are still trying to perfect the jackknife technique to estimate the minimum detectable difference in cells per nest between experimental and control areas. When the same 4

categories of cells per nest as were used on the original data are used in CATMOD analyses of the modified data sets, we are unable to detect reductions of 1, 2 or 3 cells per nest at experimental areas. We can detect a reduction of 4 cells per nest with an α of 0.05 and a power of .89, if three categories are used in the CATMOD tests: 1 cell per nest, 2 and 3 cells per nest, and 4 or more cells per nest. This minimum detectable difference is disappointingly high because variability in cells per nest is high for M. relativa. It may not be worthwhile to test for differences in cells per nest between experimental and control areas for this species. However, cells per nest are recorded automatically when cell lengths are recorded, so the data are available in case we change our minds.

CATMOD analysis of M. inermis data has not yet been accomplished. The range was 1 to 7 cells (eg., Fig. 8). We hope that a reduced variability will allow us to detect smaller differences in cells per nest between experimental and control areas than could be detected for M. relativa.

Hypothesis 2. Bees exposed to ELF EM fields, and bees not exposed, will make nest plugs of the same thickness and will devote the same proportion of nest space to reproduction.

We are currently trying to decide how best to analyze these data. The length of nest plugs will be tested first with M. inermis, since complete nests for this species usually have a solid, uninterrupted nest plug between the last reproductive cell and the nest opening. In contrast, M. relativa nests usually have empty vestibular spaces between two or more nest plugs (Fig. 1), and are thus more complex to analyze. Nest plug lengths for M. inermis are skewed in distribution (eg., Fig. 9). We hope to find a transformation that will normalize the distribution.

In analyzing the proportion of space devoted to reproduction, we wish to compare the sum of reproductive cell lengths with space in the nest used by the bee. Used space may not necessarily include the full nest depth. Basal spaces and indentations (Fig. 1) are first subtracted from total nest depth. The ratio of reproductive space to used space approaches 1.0 as the length of nest plugs and vestibular spaces decreases. We can test whether the distributions of this ratio for experimental and control areas are the same, using a Goodness of fit test. We expect to have the analysis results for the 1988 Annual Report.

Hypothesis 3. The number of leaves used to line a cell is unchanged when bees are exposed to ELF EM fields.

We counted the number of elongate leaves lining M. inermis cells from both 1985 and 1986, and 1986 M. relativa cells. Mean number of leaves per cell was about twice as large for M. inermis as for M. relativa (Table 24). Number of leaves tended to increase with cell order from base to nest entrance. It also tended to increase with bore size.

Because leaves per cell are discrete data, we expect to use the SAS CATMOD procedure, rather than the GLM procedure to test for differences between control and experimental areas.

Hypothesis 4. The relative acceptability of nests oriented in a NS direction vs. nests oriented in an EW direction does not change when bees are exposed to ELF EM fields.

The data to test this hypothesis are currently in our SAS data set. However, we have not yet had time to test this hypothesis. The results of this test will be presented in the 1988 Annual Report.

V NEST ACTIVITY RESULTS

Sample sizes.

Seven notebooks of nest activity data taken by five different observers from 1983 - 1986 have been transcribed to the computer. From these notebooks we have created a data set consisting of LO timings involved in cell cap construction. Those LO trips that were involved in nest plug construction, or in cell lining (see 1986 Annual Report) were not included in this data set. Number of bees for which we have LO trip durations at each of the four sites each year are presented in Table 25. Few bees were timed at the control sites.

During the 1987 summer season, LO trips for at least 27 M. inermis individuals were timed at each of three of the sites (Table 26). At the CL site, however, very few bees nested after July 10 (Fig. 6), so only 10 individual M. inermis were timed at this site. In the upcoming field season we will try to spend more time early in the season at the CL site than we did in 1987, in order to bring the number of bees timed to levels more closely approximating those of the other sites.

An average of 5.8 LO trips were timed for each bee in 1987 (Table 26). Usually all of these trips were part of the cell capping behavior for the same cell. Our analysis assumes that the timings for a given cell cap are independent of the timings for other cell caps. The assumption may not be strictly true for 10 cases (10%) in which the same bee was timed capping more than one cell. However, since only a small proportion of the timings fall in this category, we believe that the assumption of independence is not seriously violated.

Numbers of individual bees and numbers of LO trips timed by each of the observers at each site are presented in Table 26. Each observer's timings were not as evenly distributed over the sites as we had hoped, although they are more evenly distributed over the sites than was the case in previous years.

Fewer bees (C5=13, CL=3, F1=7, F2=6) and LO trips (C5=59, CL=17, F1=23, F2=36) were timed for M. relativa, than for M. inermis. These data are not yet analyzed, so we do not know if we have large enough samples to detect differences between experimental and control areas. Because M. relativa is active for a shorter period of time than M. inermis, and because these bees are harder to observe than M. inermis due to their small size, we may choose not to study nest activity of M. relativa in the future.

Hypothesis 5. The duration of round leaf (LO) foraging trips remains the same when bees are exposed to ELF EM fields.

During the 1987 field season we noticed that LO trip durations increased with time since the bee laid her egg. We assigned a rank order to each LO timing in a given capping sequence, although a rank of 1 is not always the first trip in the capping sequence. Thus the variance may be somewhat inflated. We should be able to improve on the situation next season.

In 1987, bees averaged 11 sec. faster at the control sites (31 sec.) than at the experimental sites (42 sec.). There was a greater difference between the two control sites than between the two experimental sites. (C5=34, CL=23, difference=11 sec.; F1=39, F2=46, difference=7 sec.)

The distributions of log LO trip durations did not differ from normal within sites and ranks in 27 out of 30 cases where more than 6 LO trips were timed (SAS univariate procedure, $\alpha=0.05$). Differences between experimental and control areas in LO trip durations were not significant (Table 27). Time of day also did not contribute significantly to variance. Sites, trip order, observers, and date were significant. About 15% of the variance was explained by this model.

The significant differences between observers in mean LO durations (FB=46, JZ=29, KR=36, KS=26, SM=41 sec., geometric means) could be due to differences in response times between observers. However, observers whose LO trip durations were the most rapid (JZ, KS) made up a greater proportion of the timings at the control areas, where bees tended to be faster. Our analysis separates differences between observers from differences between sites, but a more even distribution of observers over sites would make it easier to detect differences between experimental and control areas if they exist. We will improve on this situation in 1988 by monitoring each observer's timings at each site as they are recorded. During 1987, we only monitored the total number of timings at each site. Furthermore, if time permits we will create a training video of bees collecting LO leaves to assess differences between observers, and to help observers standardize their timings.

The data prior to 1987 were analyzed using the same GLM procedure. Because so few individuals were timed in any given year, we combined all LO trip durations over years, and did not test for differences between years. Although control sites were under-represented, we present these data for

comparison with 1987 because of the trends suggested. As in 1987, LO trip durations at control sites averaged 12 seconds faster than at experimental sites. Unlike 1987, the difference between sites was greater for experimental than control areas, although average LO trip durations had the same rank order of sites in both years (C5=29, CL=26, difference=3; F1=37, F2=48, difference=11 sec.). In the GLM procedure (Table 28), neither experimental vs. control areas, sites nested in areas, nor time of day contributed significantly to the variance in LO duration. Trip order, observer, and date were significant. These results also correspond well with the 1987 data.

Because time of day did not contribute significantly to variance in LO durations, we do not expect weather variables such as temperature and solar radiation to contribute to variability in LO durations. Weather data has not yet been added to our nest activity SAS data set, but when it is we will incorporate it in the model.

Based on our performance during the 1987 season, we estimate that future sample sizes will average about 20 bees per site, and at least 4.5 LO trips per bee. Thus we estimate a minimum of 180 LO durations each in experimental and control areas. In 1987 we timed over 290 trips in each of the two areas. Using the error mean square for sites nested in experimental areas (6.26, Table 26) we should be able to detect a 2.68 fold difference ($X=30$ vs. $X=80$ sec.) in LO durations at the experimental area for the lower sample size, and a 2.2 fold difference ($X=30$ vs. $X=65$ sec.) for the higher sample size.

VI EMERGENCE RESULTS

Hypothesis 6. Overwintering survival of megachilid bees is unchanged by exposure to ELF EM fields.

The data to test this hypothesis are currently in our SAS data set. However, they have not yet been analyzed. We expect to analyze them during the summer. We will be particularly interested at that time in comparing survival during the 1987 season, when nests were overwintered at the site where they were constructed, with survival during previous years when nests were overwintered at Channing. The 1987 data will not be available until this summer.

VII SUMMARY

Studies of the effects of high voltage transmission lines and magnetic fields in honeybees suggest several ways that solitary megachilid bees might be affected by ELF electromagnetic fields. In particular, honeybees show greater levels of activity, reduced reproductive output, lower overwintering survival and modifications of nest structure in response to high voltage transmission lines. In addition, honeybees can detect magnetic fields and may use them in orientation. ELF EM fields may affect megachilid bees in similar ways.

Megachilid bees are particularly well suited for this study. Their investment per offspring and reproductive output per nest are easy to measure because they provide each offspring with a discrete cell, and because they readily nest in artificial nests. Three types of data have been gathered in past years: nest architecture, nest activity, and emergence/mortality.

Two abundant species at the experimental and control sites, both in the genus Megachile, are the focus of our analysis. These species differ in size and degree of sexual dimorphism. Thus, they may be impacted differently by ELF EM fields.

Four hypotheses regarding the impact of ELF EM fields on nest architecture are being tested:

Hypothesis 1: The average size (length and volume) of cells for each offspring, and/or the average number of cells produced per nest is unchanged by exposure to ELF electromagnetic fields.

Hypothesis 2. Bees exposed to ELF EM fields, and bees not exposed, will make nest plugs of the same thickness and will devote the same proportion of nest space to reproduction.

Hypothesis 3. The number of leaves used to line a cell is unchanged by exposure to ELF EM fields.

Hypothesis 4. The relative acceptability of nests oriented in a NS direction vs. nests oriented in an EW direction does not change when bees are exposed to ELF EM fields.

1985 and 1986 data for both M. relativa and M. inermis have been analyzed. These data suggest that, prior to the ELF antenna becoming operational, there are no significant differences between experimental and control areas in cell

length and volume. Similarly, there is no significant interaction between experimental vs. control areas and year, in cell lengths and volumes. This means that prior to operation of the ELF antenna, cell lengths and volumes in both areas are affected equally by differences between years, or are not affected by years. Thus, we should be able to detect effects of ELF EM fields on cell lengths and volumes by a significant interaction term when the antenna is operational. The minimum number of nests that we have collected at the control sites is still sufficient to detect a 15% change in mean cell length for M. relativa (mean = 11mm) and a 5% change in mean cell length for M. inermis (mean = 15mm) with a power of 0.75 and an α of 0.05.

Number of cells per nest has been analyzed only for M. relativa. There are no significant differences between experimental and control areas, years, or the interaction between the two. However, because of the high variability in cells per nest for this species, the analysis may not be very sensitive to changes in cells per nest between experimental and control sites. We hope that it will be easier to detect differences with the less variable M. inermis data.

We have not yet analyzed the data to test hypotheses 2 and 4. Leaves lining a cell (hypothesis 3) appears to increase with size of the bore, and with proximity to the nest entrance. We have not yet tested for differences between experimental and control areas.

One hypothesis regarding nest activity is being tested:

Hypothesis 5. The duration of round leaf (LO) foraging trips remains the same when bees are exposed to ELF EM fields.

Changes in protocol begun in 1987 has greatly increased the number of bees timed for the duration of round-leaf collecting trips. Experimental vs. Control areas did not contribute significantly to the variance in LO trip duration for M. inermis. The rank order of trips in a capping sequence is a significant covariate with LO trip duration, and we must take care to record this variable in the future. If sample sizes in the future are comparable with those from 1987, we should be able to detect a 2.2 to 2.7 fold increase in LO duration (from 30 to 65 or 80 seconds) with a power of 0.75 and $\alpha = 0.05$. This magnitude of change is possible if bees are disoriented by ELF EM fields.

One hypothesis concerning emergence and mortality data has not yet been analyzed:

Hypothesis 6. Overwintering survival of megachilid bees is unchanged by exposure to ELF fields.

In the past, all nests were overwintered at Channing, but the 1987 nests overwintered at the site where they were constructed. We will be particularly interested in comparing mortality in 1987 nests with mortality in previous years.

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IX LIST OF ACRONYMS

C5: Camp 5 control site

CATMOD: Categorical data modeling procedure in SAS

CL: County Line control site

doneby: variable indicating the observer or measurer of data

ELF: Extremely Low Frequency

EM: Electromagnetic

Exp: Variable indicating whether the data were from an experimental or a control area.

Exp*year: Interaction effect of the Exp and year variables in the GLM or CATMOD model.

F1: Ford 1 (north Ford) experimental site

F2: Ford 2 (south Ford) experimental site

GLM: General Linear Modeling procedure in SAS

SAS; Statistical software package on the VAX computer, used in analysis of data

Site [exp]: Site variable nested in experimental areas

TABLE 1: Bore size categories, and diameter of drill bits associated with each category. (Number of *s indicate relative use by the two Megachile species.

Size	Diameter, mm	Used by <u>M. relativa</u>	Used by <u>M. inermis</u>
6	4.4		
2	5.2	**	
4	5.5, 6.0	***	
5	7.2	**	*
3	9.4		**
7	11.0		***

TABLE 2: Number of nests of M. relativa and M. inermis at each site for which we have data on cell lengths. (Numbers in parenthesis indicate number of hatches out of six total with more than five nests of a given species.)

Site	Control Sites		Test Sites	
	Camp 5	County Line	Ford 1 (north)	Ford 2 (south)
Year				
<u>M. relativa</u>				
1985	50 (5)	75 (6)	82 (6)	90 (6)
1986	48 (6)	48 (6)	39 (5)	82 (6)
1987	70 (5)	48 (5)	66 (4)	50 (5)
<u>M. inermis</u>				
1985	23 (3)	16 (2)	162 (6)	87 (6)
1986	15 (2)	2 (0)	44 (3)	70 (5)
1987	42 (3)	25 (3)	109 (5)	85 (6)

TABLE 3: GLM of all cells from 1985 and 1986 M. relativa nests; Cell lengths.

CELL LENGTHS				
Source of variation	df	SS	F	P>F
Year	1	2.21	2.29	0.1301
Diameter	1	56.79	58.88***	0.0001
Exp	1	2.93	0.35	0.6136
Site [exp]	2	16.70	8.66***	0.0002
Exp*year	1	2.35	0.28	0.6485
Complete vs. incomplete	1	5.93	6.15*	0.0132
Doneby	5	67.70	14.04***	0.0001
Cell order	1	51.53	53.43***	0.0001
Cells per nest	1	0.56	0.58	0.4466
Model	14	289.49	21.44***	0.0
Error	1752	1689.75		
$\bar{X} = 10.8\text{mm}$ CV = 9.1 $r^2 = 0.15$				

TABLE 4: GLM of all cells from 1985 and 1986 M. relativa nests; Cell volumes.

CELL VOLUMES

Source of variation	df	SS	F	P>F
Year	1	0.000	0.03	0.8734
Diameter	1	9.592	11203.79***	0.0
Exp	1	0.003	0.46	0.5676
Site [exp]	2	0.014	8.25***	0.0003
Exp*year	1	0.003	0.44	0.5735
Complete vs. incomplete	1	0.003	3.72	0.0538
Doneby	5	0.059	13.83***	0.0001
Cell Order	1	0.040	46.25***	0.0001
Cells per nest	1	0.002	2.61	0.1062
Model	14	10.338	862.48***	0.0
Error	1752	1.500		

$\bar{X} = 0.294\text{cm}^3$

CV = 9.9

$r^2 = 0.87$

TABLE 5: GLM of cells in bore size 4 nests, 1985 and 1986 M. relativa nests; Cell lengths.

CELL LENGTHS				
Source of variation	df	SS	F	P>F
Year	1	1.90	2.07	0.1501
Diameter	1	0.49	0.53	0.4659
Exp	1	0.27	0.02	0.9070
Site [exp]	2	31.37	17.10***	0.0001
Exp*year	1	1.50	0.10	0.7865
Complete vs. incomplete	1	5.96	6.49*	0.0109
Doneby	5	44.77	9.76***	0.0001
Cell order	1	43.45	47.38***	0.0001
Cells per nest	1	0.26	0.29	0.5926
Model	14	155.53	12.11***	0.0001
Error	1211	1110.56		
$\bar{X} = 10.9\text{mm}$ $CV = 8.8$ $r^2 = 0.12$				

TABLE 6: GLM of cells in bore size 4 nests, 1985 and 1986 M. relativa nests; Cell volumes.

CELL VOLUMES				
Source of variation	df	SS	F	P>F
Year	1	0.010	14.44***	0.0002
Diameter	1	2.011	2854.86***	0.0
Exp	1	0.002	0.18	0.7148
Site [exp]	2	0.024	16.99***	0.0001
Exp*year	1	0.005	0.43	0.5782
Complete vs. incomplete	1	0.004	5.26*	0.0220
Doneby	5	0.017	4.96***	0.0002
Cell order	1	0.028	40.30***	0.0001
Cells per nest	1	0.000	0.04	0.8416
Model	14	2.252	228.36	0.0
Error	1211	0.853		
$\bar{X} = 0.276\text{cm}^3$ $CV = 9.6$ $r^2 = 0.73$				

TABLE 7: GLM, including date of nest initiation, 1985 and 1986 M. relativa nests (except 1986 F1); Cell lengths.

CELL LENGTHS				
Source of variation	df	SS	F	P>F
Year	1	0.90	0.99	0.3210
Diameter	1	64.06	70.08***	0.0001
Exp	1	12.50	1.03	0.4173
Site [exp]	2	24.31	13.29***	0.0001
Exp*year	1	23.16	1.91	0.3015
Complete vs. incomplete	1	0.52	0.57	0.4515
Doneby	5	74.09	16.21***	0.0001
Cell order	1	44.25	48.41***	0.0001
Cells per nest	1	0.14	0.15	0.6985
Date	1	33.05	36.15***	0.0001
Model	15	322.64	23.53***	0.0
Error	1529	1397.67		
$\bar{X} = 10.8\text{mm}$ $CV = 8.9$ $r^2 = 0.19$				

TABLE 8: GLM, including date of nest initiation, 1985 and 1986 M. relativa nests (except 1986 F1); Cell volumes.

CELL VOLUMES				
Source of variation	df	SS	F	P>F
Year	1	0.004	4.27*	0.0390
Diameter	1	8.583	10230.55***	0.0
Exp	1	0.010	1.19	0.3888
Site [exp]	2	0.017	10.07***	0.0001
Exp*year	1	0.016	1.88	0.3039
Complete vs. incomplete	1	0.001	1.07	0.3006
Doneby	5	0.060	14.29***	0.0001
Cell order	1	0.035	41.75***	0.001
Cells per nest	1	0.000	0.55	0.4578
Date	1	0.006	6.99*	0.0083
Model	15	9.390	746.09***	0.0
Error	1529	1.283		
$\bar{X} = 0.294\text{cm}^3$ CV = 9.9 $r^2 = 0.88$				

TABLE 9: GLM of cells with leaf counts, 1986 M. relativa nests; Cell lengths.

CELL LENGTHS				
Source of variation	df	SS	F	P>F
Diameter	1	5.42	6.17*	0.0133
Exp	1	4.09	0.93	0.4366
Site [exp]	2	8.79	5.00*	0.0071
Complete vs. incomplete	1	4.95	5.63*	0.0180
Doneby	3	18.53	7.03***	0.0001
Cell order	1	3.72	4.24*	0.0400
Cells per nest	1	4.02	4.58*	0.0328
Leaves per cell	1	0.12	0.13	0.7176
Model	11	63.00	6.52***	0.0001
Error	516	453.16		
$\bar{X} = 11.0\text{mm}$ $CV = 8.6$ $r^2 = 0.12$				

TABLE 10: GLM of cells with leaf counts, 1986 M. relativa nests; Cell volumes.

CELL VOLUMES				
Source of variation	df	SS	F	P>F
Diameter	1	1.620	2354.13***	0.0000
Exp	1	0.005	4.73	0.1616
Site [exp]	2	0.002	1.59	0.2058
Complete vs. incomplete	1	0.001	1.24	0.2657
Doneby	3	0.010	4.68**	0.0031
Cell order	1	0.003	4.03*	0.0452
Cells per nest	1	0.003	4.93*	0.0268
Leaves per cell	1	0.008	10.93**	0.0010
Model	11	2.281	301.37***	0.0
Error	516	0.355		
$\bar{X} = 0.285\text{cm}^3$	CV = 9.2	$r^2 = 0.87$		

TABLE 11: GLM of cells for which offspring's sex is known; 1985 and 1986 M. relativa nests; Cell lengths.

CELL LENGTHS				
Source of variation	df	SS	F	P>F
Year	1	4.89	5.62*	0.0180
Diameter	1	38.84	44.70***	0.0001
Exp	1	13.07	7.35	0.1134
Site [exp]	2	3.56	2.05	0.1299
Exp*year	1	0.64	0.36	0.6088
Complete vs. incomplete	1	7.63	8.78**	0.0032
Doneby	5	19.50	4.49***	0.0005
Cell order	1	12.06	13.88***	0.0002
Cells per nest	1	0.24	0.28	0.5961
Sex	1	23.65	27.22***	0.0001
Model	15	156.12	11.98***	0.0001
Error	646	561.28		
$\bar{X} = 10.9\text{mm}$ $CV = 8.6$ $r^2 = 0.22$				

TABLE 12: GLM of cells for which offspring's sex is known; 1985 and 1986 M. relativa nests; Cell volumes.

CELL VOLUMES				
Source of variation	df	SS	F	P>F
Year	1	0.001	1.86	0.1728
Diameter	1	2.704	3883.10***	0.0
Exp	1	0.011	17.16	0.0536
Site [exp]	2	0.001	0.93	0.3943
Exp*year	1	0.000	0.04	0.8680
Complete vs. incomplete	1	0.005	7.45*	0.0065
Doneby	5	0.019	5.34***	0.0001
Cell Order	1	0.010	14.78***	0.0001
Cells per nest	1	0.000	0.28	0.5975
Sex	1	0.019	27.30***	0.0001
Model	15	3.224	308.66***	0.0
Error	646	0.450		
$\bar{X} = 0.293\text{cm}^3$ CV = 9.0 $r^2 = 0.89$				

TABLE 13: Differences between observers in mean cell lengths and cell volumes (M.relativa, bore size 4 only).

Measurer (Doneby)	Mean Cell Lengths mm	Mean Cell Volumes cm ³	No. Cells Measured
ER (1985)	10.90	0.269	194
JZ (1986)	11.18	0.268	171
KS (1985,86)	11.06	0.281	399
LS (1986)	10.67	0.293	145
MS (1986)	10.85	0.271	79
ND (1985)	10.62	0.269	238

TABLE 14: Mean cell length by cell order in the nest.
Basal cell = C1.

Cell order	<u>M. relativa</u>			<u>M. inermis</u>		
	N	\bar{X}	SD	N	\bar{X}	SD
C1	309	11.3	1.1	249	15.7	1.7
C2	253	10.9	1.1	220	15.2	1.5
C3	197	10.8	0.9	190	15.0	1.4
C4	149	10.7	1.0	152	15.1	1.3
C5	109	10.7	0.9	82	15.0	1.5
C6	77	10.7	0.8	17	14.9	1.0
C7	62	10.7	0.9	1	13.9	
C8	39	10.6	0.9			
C9	26	10.4	0.8			
C10	8	10.0	0.6			
C11	2	10.3	1.3			
C12	1	10.2				

TABLE 15: GLM of all cells, 1985 and 1986 M. inermis nests;
Cell lengths.

CELL LENGTHS				
Source of variation	df	SS	F	P>F
Year	1	1.01	0.53	0.4687
Diameter	1	37.61	19.59***	0.0001
Exp	1	11.95	4.65	0.1639
Site [exp]	2	5.14	1.34	0.2624
Exp*year	1	13.19	5.13	0.1518
Complete vs. incomplete	1	24.55	12.79***	0.0004
Doneby	3	161.84	28.10***	0.0001
Cell order	1	61.46	32.01***	0.0001
Cells per nest	1	6.84	3.56	0.0592
Leaves per cell	1	36.18	18.84***	0.0001
Model	13	501.33	20.09***	0.0
Error	1312	2519.01		
$\bar{X} = 15.1\text{mm}$ $CV = 9.2$ $r^2 = 0.17$				

TABLE 16: GLM of all cells, 1985 and 1986 M. inermis nests;
Cell volumes.

CELL VOLUMES				
Source of variation	df	SS	F	P>F
Year	1	0.012	0.80	0.3719
Diameter	1	82.162	5567.26***	0.0
Exp	1	0.099	58.41*	0.0167
Site [exp]	2	0.003	0.12	0.8911
Exp*year	1	0.033	19.48*	0.0477
Complete vs. incomplete	1	0.108	7.31*	0.0069
Doneby	3	1.286	29.04***	0.0001
Cell order	1	3.494	23.67***	0.0001
Cells per nest	1	0.182	12.33***	0.0005
Leaves per cell	1	0.024	1.59	0.2070
Model	13	104.419	544.26***	0.0
Error	1312	19.363		
$\bar{X} = 1.27\text{cm}^3$	CV = 9.6	$r^2 = 0.84$		

TABLE 17: GLM of cells in bore size 7 nests, 1985 and 1986 M. inermis nests; Cell lengths.

CELL LENGTHS				
Source of variation	df	SS	F	P>F
Year	1	4.12	2.17	0.1413
Diameter	1	24.63	12.96***	0.0003
Exp	1	3.36	2.15	0.2800
Site [exp]	2	3.12	0.82	0.4398
Exp*year	1	0.52	0.33	0.6232
Complete vs. incomplete	1	16.47	8.66**	0.0033
Doneby	3	168.88	29.62***	0.0001
Cell order	1	30.06	15.82***	0.0001
Cells per nest	1	25.53	13.43***	0.0003
Leaves per cell	1	13.55	7.13*	0.0077
Model	13	350.08	14.17***	0.0001
Error	824	1565.91		
$\bar{X} = 15.2\text{mm}$ $CV = 9.0$ $r^2 = 0.18$				

TABLE 18: GLM of cells in bore size 7 nests, 1985 and 1986 M. inermis nests; Cell volumes.

CELL VOLUMES				
Source of variation	df	SS	F	P>F
Year	1	0.034	2.10	0.1479
Diameter	1	18.280	1127.20***	0.0
Exp	1	0.035	3.19	0.2162
Site [exp]	2	0.022	0.67	0.5110
Exp*year	1	0.002	0.17	0.7183
Complete vs. incomplete	1	0.118	7.26*	0.0072
Doneby	3	1.314	27.00***	0.0001
Cell order	1	0.249	15.38***	0.0001
Cells per nest	1	0.199	12.27***	0.0005
Leaves per cell	1	0.117	7.23*	0.0073
Model	13	24.053	114.09***	0.0
Error	824	13.363		
$\bar{X} = 1.39\text{cm}^3$ CV = 9.1 $r^2 = 0.64$				

TABLE 19: GLM, including date of nest initiation, 1985 and 1986 M. inermis nests (except 1986 F1); Cell lengths.

CELL LENGTHS				
Source of variation	df	SS	F	P>F
Year	1	0.00	0.00	0.9818
Diameter	1	6.22	3.32	0.0688
Exp	1	5.27	1.15	0.3950
Site [exp]	2	9.12	2.43	0.0883
Exp*year	1	3.19	0.70	0.4911
Complete vs. incomplete	1	17.79	9.49**	0.0021
Doneby	3	101.06	17.96***	0.0001
Cell order	1	60.51	32.27***	0.0001
Cells per nest	1	15.81	8.43**	0.0038
Leaves per cell	1	67.38	35.93***	0.0001
Date	1	15.67	8.36**	0.0039
Model	14	401.06	15.28***	0.0001
Error	1108	2077.98		
$\bar{X} = 15.0\text{mm}$ $CV = 9.1$ $r^2 = 0.16$				

TABLE 20: GLM, including date of nest initiation, 1985 and 1986 M. inermis nests (except 1986 F1); Cell volumes.

CELL VOLUMES				
Source of variation	df	SS	F	P>F
Year	1	0.010	0.67	0.4148
Diameter	1	68.909	4535.68***	0.0
Exp	1	0.066	11.82	0.0752
Site [exp]	2	0.011	0.37	0.6941
Exp*year	1	0.022	4.02	0.1827
Complete vs. incomplete	1	0.080	5.28*	0.0217
Doneby	3	0.736	16.15***	0.0001
Cell order	1	0.346	22.78***	0.0001
Cells per nest	1	0.199	13.11***	0.0003
Leaves per cell	1	0.093	6.13*	0.0135
Date	1	0.037	2.40	0.1214
Model	14	88.967	418.28***	0.0
Error	1108	16.833		
$\bar{X} = 1.27\text{cm}^3$ CV = 9.7 $r^2 = 0.84$				

TABLE 21: GLM of cells for which offspring's sex is known; 1985 and 1986 M. inermis nests; Cell lengths.

CELL LENGTHS				
Source of variation	df	SS	F	P>F
Year	1	0.76	0.56	0.4560
Diameter	1	12.78	9.34**	0.0024
Exp	1	0.20	0.03	0.8775
Site [exp]	2	12.88	4.71*	0.0096
Exp*year	1	0.35	0.05	0.8369
Complete vs. incomplete	1	7.43	5.43*	0.0204
Doneby	3	84.61	20.62***	0.0001
Cell order	1	17.94	13.11***	0.0003
Cells per nest	1	17.68	12.93***	0.0004
Leaves per cell	1	4.11	3.00	0.0839
Sex	1	14.48	10.59**	0.0013
Model	14	252.99	13.21***	0.0001
Error	347	474.65		
$\bar{X} = 15.2\text{mm}$	CV = 7.7	$r^2 = 0.35$		

TABLE 22: GLM of cells for which offspring's sex is known; 1985 and 1986 M. inermis nests; Cell volumes.

CELL VOLUMES				
Source of variation	df	SS	F	P>F
Year	1	0.009	0.83	0.3634
Diameter	1	12.732	1163.74***	0.0
Exp	1	0.006	0.11	0.7739
Site [exp]	2	0.116	5.29*	0.0055
Exp*year	1	0.003	0.05	0.8505
Complete vs. incomplete	1	0.059	5.41*	0.0206
Doneby	3	0.569	17.34***	0.0001
Cell order	1	0.126	11.48**	0.0008
Cells per nest	1	0.127	11.64**	0.0007
Leaves per cell	1	0.030	2.74	0.0985
Sex	1	0.118	10.79**	0.0011
Model	14	22.455	146.60***	0.0
Error	347	3.796		
$\bar{X} = 1.30\text{cm}^3$ CV = 8.1 $r^2 = 0.86$				

TABLE 23: Categorical modeling of number of cells per complete nest of Megachile relativa.

Source of variance	df	Chi.Square	Prob.
Intercept	3	8.63	0.0347*
Exp	3	7.63	0.0543
Site [exp]	6	18.63	0.0048**
Year	3	0.81	0.8478
Exp*year	3	4.29	0.2315
Likelihood Ratio	6	9.85	0.1313

TABLE 24: Mean number of leaves lining cells, by cell order in the nest, and by bore size. Basal cell = C1.

Cell order	<u>M. relativa</u>			<u>M. inermis</u>		
	N	\bar{X}	SD	N	\bar{X}	SD
C1	106	6.6	1.8	232	12.9	3.2
C2	97	6.8	2.1	206	12.6	3.0
C3	79	6.5	1.6	181	12.7	3.3
C4	59	6.6	1.7	142	13.6	3.9
C5	44	6.8	1.8	74	14.6	4.4
C6	29	6.8	1.6	13	15.8	5.8
C7	21	7.0	1.5			
C8	11	6.4	1.2			
C9	6	7.3	1.8			
C10	3	8.7	0.6			
C11	2	5.5	2.1			
Bore Size						
2	79	6.3	1.6			
4	457	6.7	1.8			
5	47	10.3	2.4	40	9.8	3.1
3				422	9.6	2.5
7				848	13.1	3.5

TABLE 25: Number of individual bees of M. inermis and number of LO trip durations timed by each observer at each site, 1983-1986.

	Control Sites		Test Sites		
Observers	Camp 5	County Line	Ford 1 (north)	Ford 2 (south)	Totals
Number of bees timed:					
1983					
AP			1	4	5
KG			6		6
1984					
JH		2	4	3	9
KG			1		1
VS			5		5
1986					
PW	2		7	6	15
Totals	2	2	24	13	41
Numbers of LO trips timed:					
1983					
AP			10	26	36
KG			43		43
1984					
JH		13	46	27	86
KG			9		9
VS			55		55
1986					
PW	26		38	55	119
Totals	26	13	201	108	348
Average no. trips per bee:					
	13.0	6.5	8.4	8.3	8.5

TABLE 26: Number of individual bees of M. inermis and number of LO trip durations timed by each observer at each site, 1987.

	Control Sites		Test Sites		
Observers	Camp 5	County Line	Ford 1 (north)	Ford 2 (south)	Totals
Number of bees timed:					
FB	8		6	3	17
JZ	9	3	7	3	22
KR	13	2	12	7	34
KS	2	2	1		5
SM	5	3	12	11	31
Totals	37	10	38	24	109
Numbers of LO trips timed:					
FB	34		22	11	67
JZ	46	22	27	20	115
KR	102	13	88	34	237
KS	14	9	4		27
SM	21	31	66	66	184
Totals	217	75	207	131	630
Average no. trips per bee:					
	5.9	7.5	5.4	5.4	5.8

TABLE 27: GLM of log transformed LO trip durations for M. inermis, 1987.

Source of variation	df	SS	F	P>F
Exp	1	19.6	3.13	0.2191
Site [exp]	2	12.5	14.10***	0.0001
Trip rank	1	13.5	30.50***	0.0001
Doneby	4	7.4	4.15**	0.0025
Time of day	1	0.6	1.31	0.2533
Time*Time	1	0.7	1.49	0.2226
Date	1	1.8	4.12*	0.0427
Model	11	48.7	9.98***	0.0001
Error	618	274.3		
$\bar{X} = 3.6$ (36 sec.) $CV = 18.6$ $r^2 = 0.15$				

TABLE 28: GLM of log transformed LO trip durations for M. inermis, 1983 - 1986.

Source of variation	df	SS	F	P>F
Exp	1	1.1	0.82	0.4615
Site [exp]	2	2.7	2.44	0.0892
Trip rank	1	25.5	46.73***	0.0001
Doneby	3	7.1	4.31**	0.0054
Time of day	1	0.0	0.18	0.6704
Time*Time	1	0.0	0.02	0.8828
Date	1	15.3	28.22***	0.0001
Model	10	60.1	11.04***	0.0
Error	301	163.9		
$\bar{X} = 3.6$ (31 sec.) CV = 20.3 $r^2 = 0.27$				

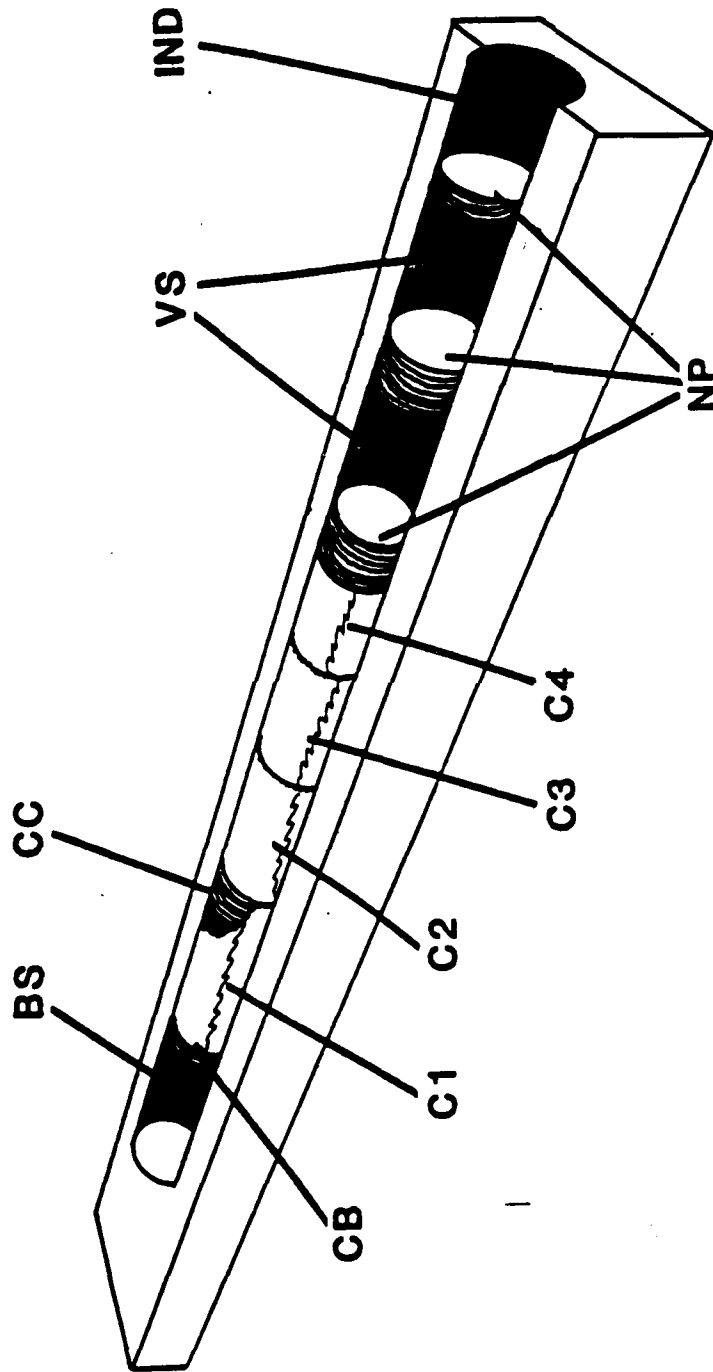


Figure 1. Cut away view of a completed Megachile nest.

BS - Basal Space; CB - Cell Base; C1, C2, C3, C4 - Reproductive Cells 1 through 4; CC - Cell Cap; NP - Nest Plug; VS - Vestibular Spaces; IND - Indentation.

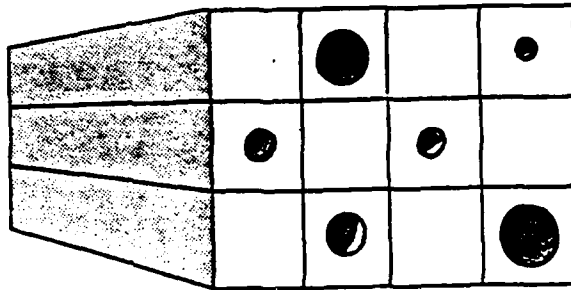


Figure 2. Arrangement of nests in block, 1983 - 1986.

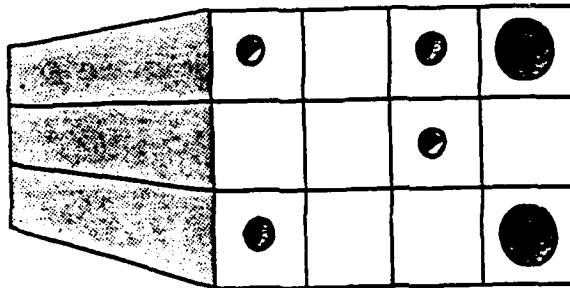


Figure 3. Arrangement of nests in block, 1987.

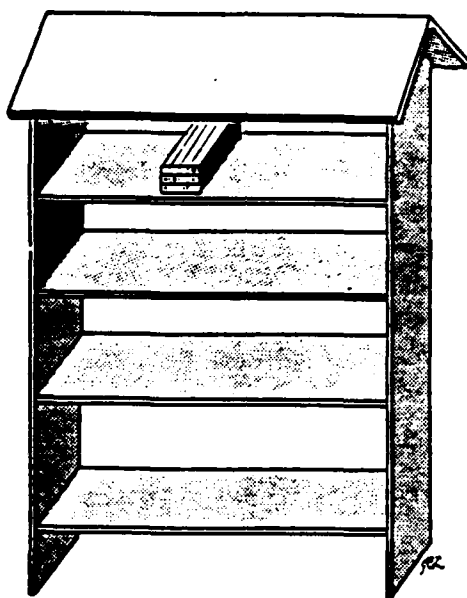
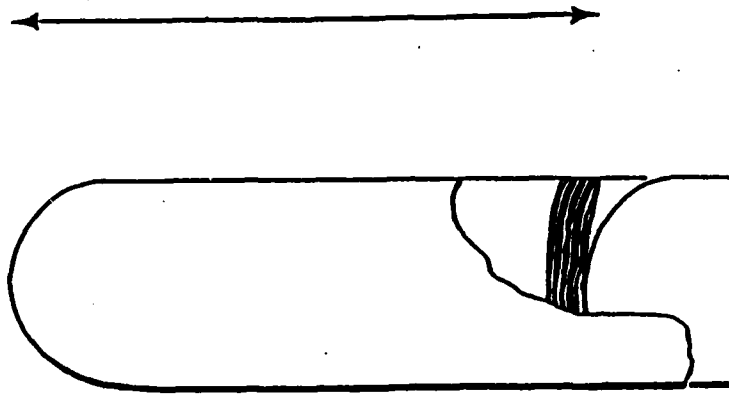


Figure 4. Hutch, with one block of nests.



↔ Cell Length Including Cap Length

Figure 5. A single reproductive cell, indicating how cell lengths are measured.

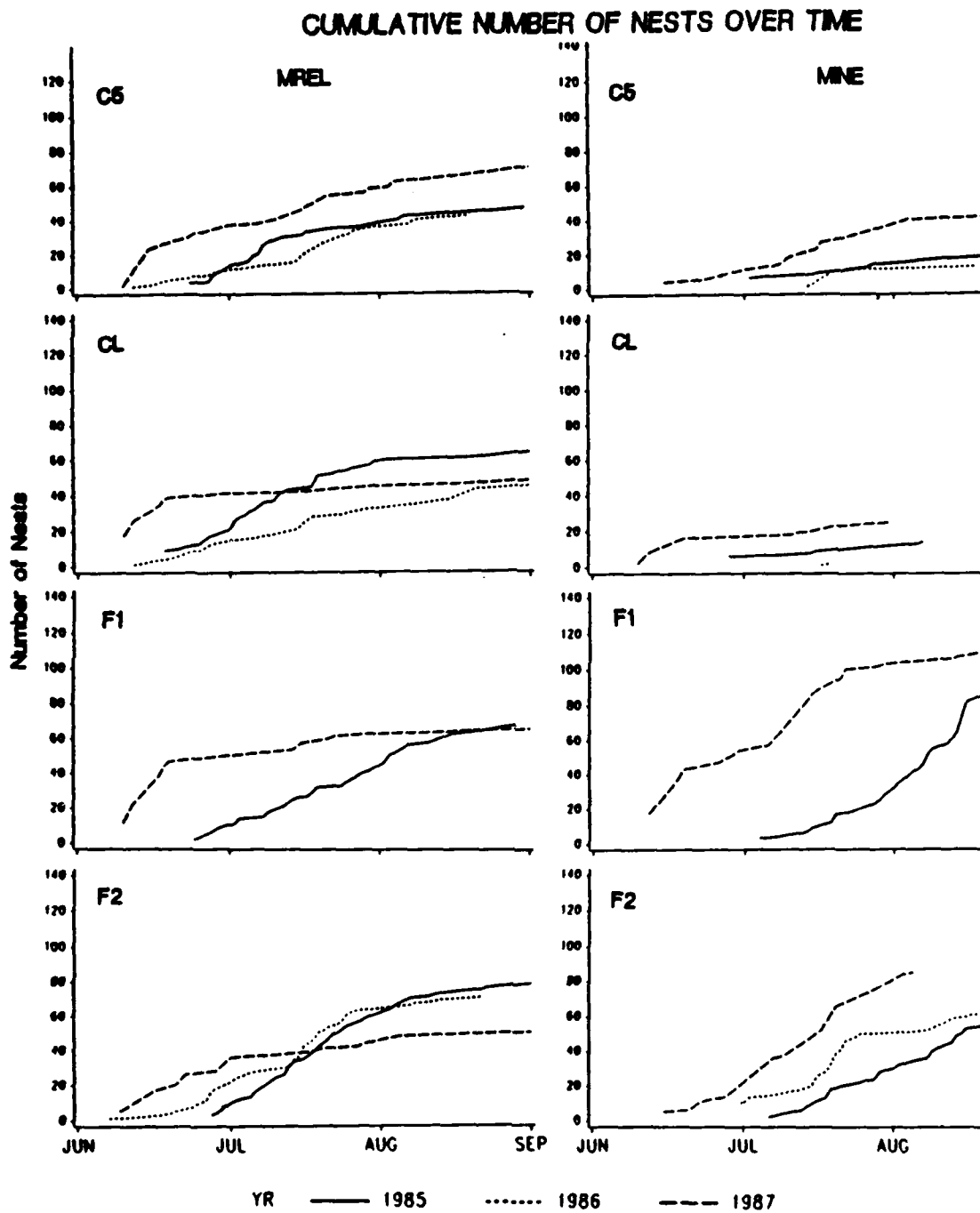


Figure 6. Cumulative number of nests of M. relativa and M. inermis at each site, 1985-1987.

SPECIES-MREL SITE-F2

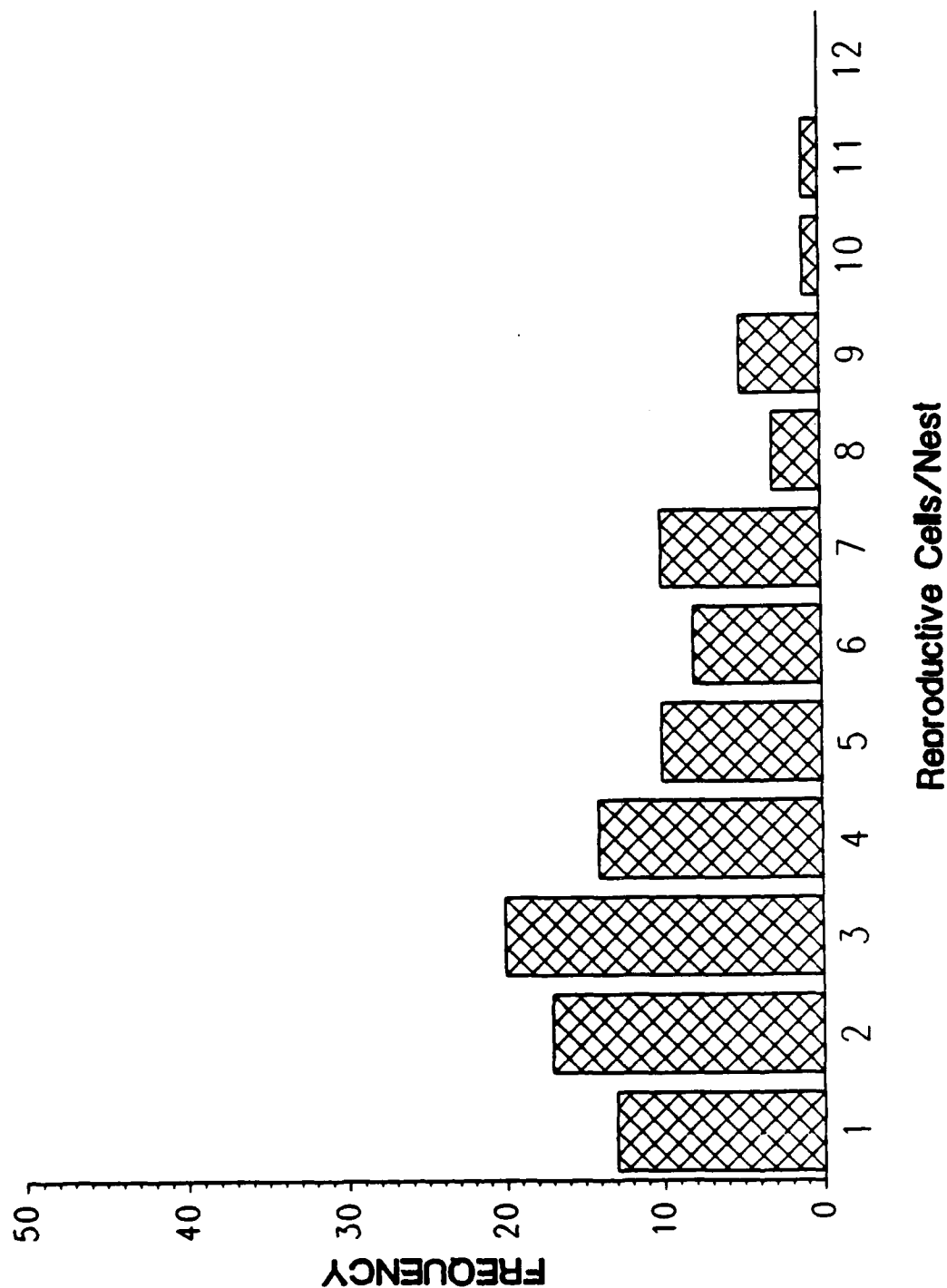


Figure 7. Distribution of reproductive cells per nest for *M. relativa* at the F2 site.

SPECIES-MNE SITE-F2

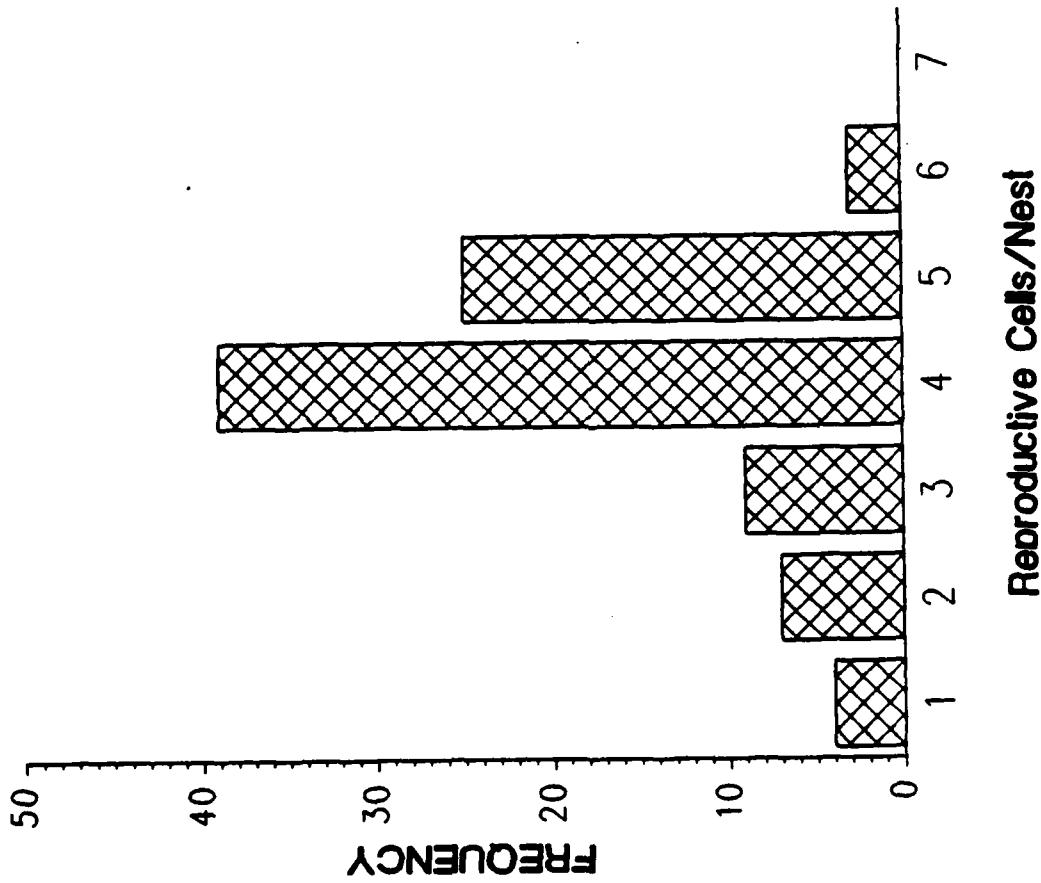


Figure 8. Distribution of reproductive cells per nest for *M. inermis* at the F2 site.

Nest Plug Length Distribution SPECIES=MINE YR=1985

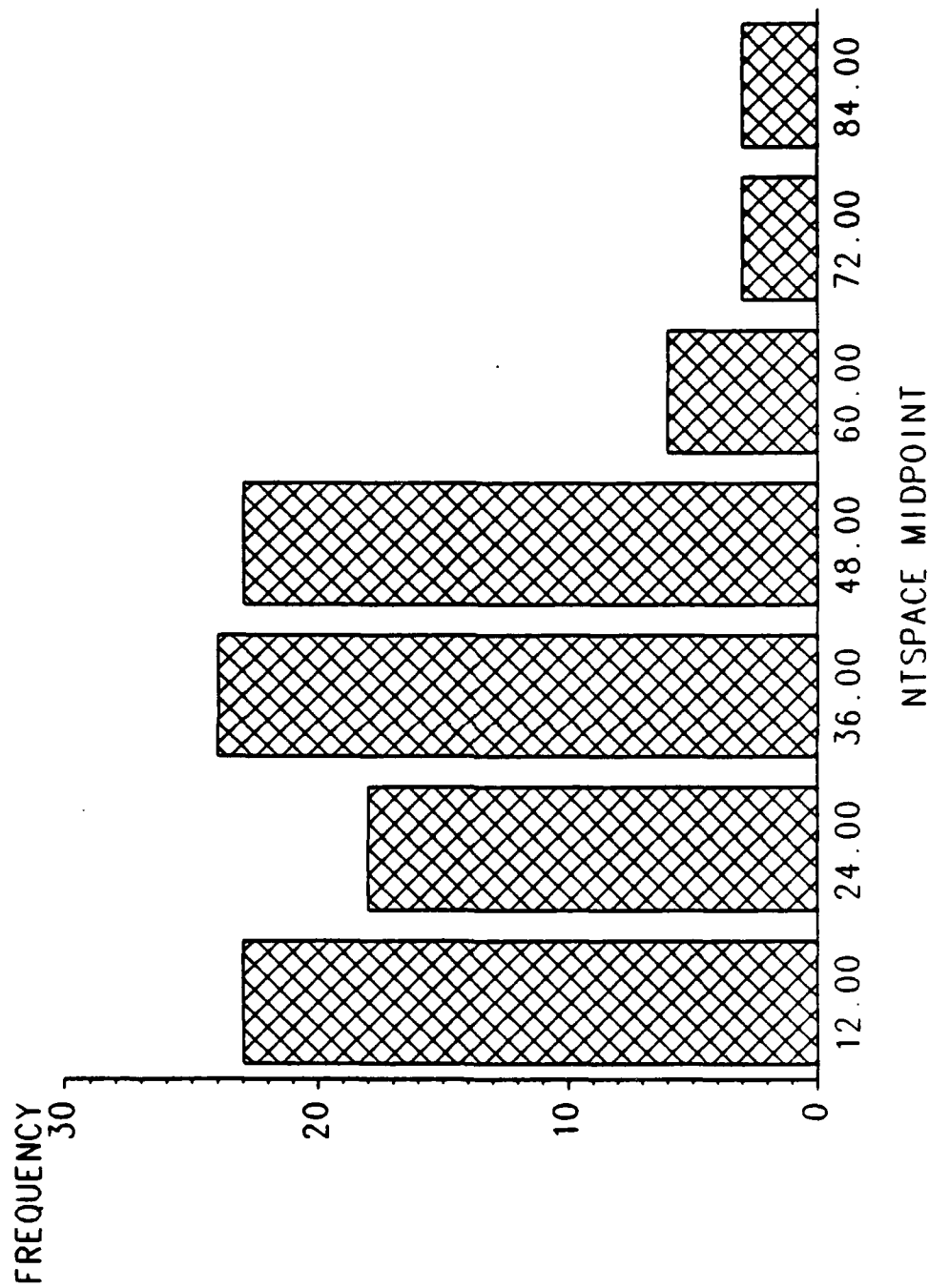


Figure 9. Distribution of nest plug lengths for M. inermis.

ELF COMMUNICATIONS SYSTEM ECOLOGICAL MONITORING PROGRAM

**SMALL VERTEBRATES: THE MICHIGAN STUDY SITE
TASKS 5.6, SMALL MAMMALS, AND 5.12A, NESTING BIRDS**

ANNUAL REPORT : 1987

Subcontract No.: E06549-84-C-006

Subcontracted to:

THE BOARD OF TRUSTEES, MICHIGAN STATE UNIVERSITY

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SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

TABLE OF CONTENTS

List of Tables.	vi
List of Figures	xii
Abstract.	xiii
Summary.	xv
Preface	1
RATIONALE OF STUDIES.	2
Behavioral Studies.	4
Reproduction, Growth and Development.	5
Maximal Aerobic Metabolism	6
COMMENTS ON AMBIENT MONITORING.	8
OVERALL RESEARCH DESIGN AND SUPPORT FACILITIES.	9
Preliminary Measurements on 60 and 76 Hz fields	13
Study of Small Mammal Communities	
I. Purpose	18
II. Methods	18
III. Results.	21
PARENTAL AND NESTLING BEHAVIOR, AND FECUNDITY, GROWTH AND MATURATION STUDIES - TREE SWALLOWS	
I. Purpose.	24
II. Methods.	24
III. Results - 1987	28
PARENTAL AND NESTLING BEHAVIOR, AND FECUNDITY, GROWTH, AND MATURATION STUDIES - DEERMICE	
I. Purpose.	39
II. Methods.	39
III. Results - 1987.	41
HOMING STUDIES - TREE SWALLOWS	
I. Purpose.	43
II. Methods.	43
III. Results - 1987.	44
HOMING STUDIES- SMALL MAMMALS	
I. Purpose	46
II. Methods.	47
III. Results - 1987.	49
DEVELOPMENTAL STUDIES	
I. Purpose	51
II. Methods.	51
STUDIES OF MAXIMUM AEROBIC METABOLISM	
I. Purpose	55
II. Methods.	55
III. Results - 1987.	61
LITERATURE CITED.	67
APPENDIX A - TABLES AND FIGURES	77

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

LIST OF TABLES

	Page
Table 1. Test-control plot pairings for the various work elements for small mammals and nesting birds. Plot code designations are those used by IITRI	79
Table 2. Minimum sample size requirements estimated for various study elements to meet the statistical standard of 90% certainty of detecting a 20% change at the 5% level of significance. The procedure follows Sokal and Rohlf (1981, pg 247) for parametric statistics and Gill (1978, pg 82) for frequencies	80
Table 3. Summary of mammal community variables at test (Pirilot Road) and control (Michigamme) sites, 1987.	81
Table 4a. Estimates of trappable population number (TPN) of chipmunks and deermice at test (Pirilot Road) and control (Michigamme) sites for years 1987, 1986 and 1985.	82
Table 4b. Electromagnetic field strengths measured by IITRI from 1983 to the present. Data from 1983 through 1985 are pre-antenna operation, 1986 and 1987 represent initial testing at 4 and 6 amperes on variable schedules (see Appendix B). Values for 1987 are from closest antenna leg	82
Table 5. Tree swallow plots, number of boxes, and percent with egg laying activity on test and control sites for 1985, 1986, and 1987. Egg laying activity is defined as at least two eggs laid before abandonment or continuation of nesting	83
Table 6. Tree swallow fecundity data for years 1987, 1986 and 1985. Data are from the Pirilot Road test plot and Tachycineta Meadows control plot and excludes any renests which may have occurred.	84
Table 7. Likelihood to hatch and fledge for tree swallows in 1987, 1986 and 1985. Data are from the Pirilot Road test plot and Tachycineta Meadows control plot. Comparisons were made using the G test of independence (df=5).	85
Table 8a. ANOVA for clutch size of tree swallows. Tested are the effects of PLOT (test and control), YEAR (1985, 1986, 1987) and the interaction of PLOT and YEAR.	86
Table 8b. ANOVA for hatch success of tree swallows. Tested are the effects of PLOT (test and control), YEAR (1985, 1986, 1987) and the interaction of PLOT and YEAR.	86
Table 8c. ANOVA for fledging success of tree swallows. Tested are the effects of PLOT (test and control), YEAR (1985, 1986, 1987) and the interaction of PLOT and YEAR.	86

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 9a. Age in days at landmark events of eye opening and primary feather eruption in 1987 and 1986. Data are from the Pirlot Road test plot and Tachycineta Meadows control plot. Sample sizes are numbers of individual young. Day of hatching is defined as day zero.	87
Table 9b. Nested ANOVA for age of eye opening in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987 and 1986. . . .	87
Table 9c. Nested ANOVA for primary feather eruption in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987 and 1986. . . .	87
Table 10. Exposure data and frequency of mortality for eggs, nestlings, and nests in 1987 calculated using the Mayfield method (Mayfield 1961, 1975). Data are pooled from all test and control plots. Comparisons between test and control were calculated using G-tests (Sokal and Rohlf 1981).	88
Table 11a. Nested ANOVA for weight increase in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987, 1986 and 1985 (logistic model used for growth constant)	89
Table 11b. Nested ANOVA for the inflection point of weight increase in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987, 1986 and 1985 (logistic model used for growth constant) .	89
Table 12a. Nested ANOVA for tarsus growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987, 1986 and 1985 (logistic model used for growth constant)	90
Table 12b. Nested ANOVA for the inflection point of tarsus growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987, 1986 and 1985 (logistic model used for growth constant)	90
Table 13a. Nested ANOVA for ulna growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987, 1986 and 1985 (logistic model used for growth constant)	91
Table 13b. Nested ANOVA for the inflection point of ulna growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987, 1986 and 1985 (logistic model used for growth constant)	91

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 14. Nested ANOVA for wing growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987, 1986 and 1985 (exponential model used for growth constant).	92
Table 15a. Tree swallow growth constants derived from fitted growth curves. Data are from test (Pirlot Road) and control (Tachycineta Meadows) sites for years 1987, 1986 and 1985 . . .	93
Table 15b. Tree swallow inflection points derived from fitted growth curves. Data are from test (Pirlot Road) and control (Tachycineta Meadows) sites for years 1987, 1986 and 1985 . . .	93
Table 16a. Minimum detectable differences and power for tree swallow growth constants derived from fitted growth curves for years 1987, 1986 and 1985	94
Table 16b. Minimum detectable differences and power for tree swallow inflection points derived from fitted growth curves for years 1987, 1986 and 1985	94
Table 17a. Nested ANOVA for incubation egg temperature of tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987.	95
Table 17b. Analysis of Covariance of mean egg temperature (x _C) for control and test birds using mean ambient temperature as a covariate	95
Table 18a. Statistics for growth rates for deermice young compared by year and plot	96
Table 18b. Nested ANOVA of deermice growth rates on test (Pirlot Road) and control (Michigamme) sites for years 1987 and 1986. Tested are the effects of plots ,PLOT, and litters within a plot, MOTHER(PLOT)	96
Table 19. Minimum detectable differences and power for deermice growth constants for years 1987 and 1986	97
Table 20a. Relevant statistics for age of eye-opening and incisor eruption for deermice reared in enclosures during 1987 and 1986.	98
Table 20b. Nested ANOVA of deermice age of eye opening on test (Pirlot Road) and control (Michigamme) sites for years 1987 and 1986. Tested are the effects of plots, PLOT, and litters within a plot, MOTHER(PLOT)	98
Table 20c. Nested ANOVA of deermice incisor eruption on test (Pirlot Road) and control (Michigamme) sites for years 1987 and 1986. Tested are the effects of plots, PLOT, and litters within a plot, MOTHER(PLOT)	98

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 21a. Results of the 1987 and 1986 tree swallow homing study. Data are from Panola Plains control plot and pooled from Cleveland Homestead and North Turner test plots. All times are in minutes from release. Returns are those birds which returned to the nesting area in less than 300 minutes. Likelihood to return was assessed using the Chi-squared statistic	99
Table 21b. Mean return times (minutes) of tree swallows compared with a t-test using pooled standard deviations	99
Table 21c. ANOVA of tree swallow return times for PLOT (test and control), YEAR (1987 and 1986) and the interaction of PLOT and YEAR.	99
Table 22. Numbers of tree swallows used in homing tests categorized by gender and age	100
Table 23. Results of the small mammal homing studies at Pirlot Road test site and Michigamme control site during the summer of 1987	101
Table 24a. Chi-square analysis of tree swallow embryo data from control plots for 1985, 1986, and 1987	102
Table 24b. Chi-square analysis of tree swallow embryo data from test plots for 1985, 1986 and 1987	103
Table 25. Chi-square analysis of pooled tree swallow embryo data from sites other than 1987 TMC, 1986 FNT and 1987 FNT . .	104
Table 26. Frequency of abnormal tree swallow embryos from plots not included in the pooled chi-square analysis compared to the pooled frequency	104
Table 27. Peak aerobic metabolic rates in deermice and chickadees captured at the Pirlot test plot (PRT) and Michigamme control plot (MGE) during the winter of 1987	105
Table 28. Summary of data on mean peak weight-specific rates of aerobic metabolism in 1985, 1986, and 1987. All data for 1985 were gathered on animals from test sites. The data listed for 1986 and 1987 are pooled across test and control sites. . .	106
Table 29 . Mean values for 60 Hz transverse electric fields (V/m) on test and control plots for years 1983 to 1987. The values in parentheses are the sample n. Values listed by IITRI as <0.001 are treated as equal to 0.001. Plot 1D3 is the release site for tree swallows used in homing studies on control plots, and plots 1D1 and 1D2 are release sites used for test plots.	107

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 30. Mean values for 60 Hz longitudinal electric fields (mV/m) on test and control plots for years 1983 to 1987. The values in parentheses are the sample n. Plot 1D3 is the release site for tree swallows used in homing studies on control plots, and plots 1D1 and 1D2 are release sites used for test plots. 108

Table 31. Mean values for 60 Hz magnetic fields (mG) on test and control plots for years 1983 to 1987. The values in parentheses are the sample n. Values listed by IITRI as <0.001 are treated as equal to 0.001. Plot 1D3 is the release site for tree swallows used in homing studies on control plots, and plots 1D1 and 1D2 are release sites used for test plots 109

Table 32. Comparison of mean values for 60 Hz fields on test and control plots averaged over the years 1983 - 1987. Fields are coded as T - transverse fields (V/m), L - longitudinal fields (mV/m) and M - magnetic fields (mG). 110

Table 33. Comparison of values for 60 Hz fields on test and control plots and the corresponding release plots used for tree swallow homing in 1986 and 1987. Fields are coded as T - transverse fields (V/m), L - longitudinal fields (mV/m) and M - magnetic fields (mG). 110

Table 34. Mean values for 76 Hz transverse electric fields (V/m) on test and control plots for years 1986 (4 or 6 amperes) and 1987 (15 amperes). The value in parentheses is the sample N. NS refers to the north-south antenna segment; EW to the averaged east-west segments in 1986 and a single measurement for simultaneous operation of both east-west segments in 1987. All measures on test plots were made on the NS segment, except for displacement plots 1D1 and 1D2, which are located north of the northernmost EW segment. All values reported by IITRI as <0.001 were set to 0.001. 111

Table 35. Mean values for 76 Hz longitudinal electric fields on test and control plots for years 1986 (4 or 6 amperes) and 1987 (15 amperes). The value in parentheses is the sample N. NS refers to the north-south antenna segment; EW to the averaged east-west segments in 1986 and a single measurement for simultaneous operation of both east-west segments in 1987. All measures on test plots were made on the NS segment, except for displacement plots 1D1 and 1D2, which are located north of the northernmost EW segment. All values reported by IITRI as <0.001 were set to 0.001. 112

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 36. Mean values for 76 Hz magnetic fields (mG) on test and control plots for years 1986 (4 or 6 amperes) and 1987 (15 amperes). The value in parentheses is the sample N. NS refers to the north-south antenna segment; EW refers to the averaged east-west segments in 1986 and a single measurement for simultaneous operation of both east-west segments in 1987. All measures on test plots were made on the NS segment, except for displacement plots 1D1 and 1D2, which are located north of the northernmost EW segment. All values reported by IITRI as <0.001 were set to 0.001 113

Table 37. Comparison of mean values for 76 Hz fields on test and control plots averaged over the years 1986 and 1987. Fields are coded as T - transverse fields (V/m), L - longitudinal fields (mV/m) and M - magnetic fields (mG). NS refers north-south antenna segment and EW refers to the two east-west segments. See Table for samples sizes for means. . 114

Table 38. Comparison of values for 76 Hz fields on test and control plots and the corresponding release plots used for tree swallow homing in 1986 and 1987. Fields are coded as T - transverse fields (V/m), L - longitudinal fields (mV/m) and M - magnetic fields (mG). Values are averaged for NS and EW antenna segment operation 115

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

LIST OF FIGURES

	Page
Figure 1. Control and test plots in relation to the ELF Communication System in Michigan. Control plots as referred to in the text are: MGE - Michigamme (North and South parts), PPC - Panola Plains, TMC - Tachycineta Meadows. Test plots are: CHT - Cleveland Homestead, NTT - North Turner; FNT - Ford North, FST - Ford South, PRT - Pirlot Road. FW is Floodwood work plot which was used in the past for tree swallow studies on embryology and homing. Also shown are the release sites used for tree swallow currently	116
Figure 2. Antenna operation log by antenna leg. Legs are designated as north-south (N-S), northern east-west (E-W 1), and southern east-west (E-W 2). Data provided by IITRI	117
Figure 3. Operation schedule and duration during 1986. Indicated below the date is the research activity in progress. Codes indicate: A - small mammal community studies, B - tree swallow, B - tree swallow nesting, egg-laying, embryology, hatching, growth and fledging studies. C - deermouse growth and parental care studies, D - tree swallow homing, E - small mammal homing	118
Figure 4. Operation schedule and duration during 1987. Indicated below the date is the research activity in progress. Codes indicate: A - small mammal community studies, B - tree swallow, B - tree swallow nesting, egg-laying, embryology, hatching, growth and fledging studies. C - deermouse growth and parental care studies, D - tree swallow homing, E - small mammal homing	119

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

ABSTRACT OF 1987 REPORT

The 1987 report contains information on base-line data preceeding antenna testing (1983 to 1985) and low amperage testing years of 1986 and 1987. While findings must be considered as incomplete until the antenna is fully operational, each years' data is useful in establishing trends in the aspects of small mammal and nesting bird biology at the study sites. Small mammal community studies revealed a lower diversity of species and abundances on the test plot in 1987. In previous years, test and control plot did not differ in diversity and abundances. Population size of eastern chipmunks was greater on the controls in 1987, a continuation of the pattern found since the study began. Deermice were more abundant on the test plot in 1987, whereas in previous years there were no differences between test and control plots. Studies of fecundity, survival, growth and parental care of tree swallows indicated clutch size was smaller on test plots in 1987 whereas the reverse was true in previous years. Hatching success was not different between plots in 1987, whereas in past years success was always greater on test plots. Egg mortality was higher in test plots in all years, as was the number of nests failing. Fledging success was not different between plots for any year except 1985 when greater success occurred on test plots. Significant interactions were noted between the year and plot, however. Age at eye opening and feather eruption were not different for test and control plots in any year. Growth rates for body mass, length of the tarsus, ulna and wing were the same for test and control plots. Parental care in warming the eggs during incubation was the same on test and control plots. Growth rates in deermice were not different between test and control plots. Age at

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

eye opening and eruption of incisors were not different on test and control plots. Homing of tree swallows to their nests after displacement 30 km took significantly longer and fewer birds returned on control than on test plots, continuing a trend noticed in 1986, but not in earlier years. Birds displaced from test plots have returned at the same rate and time in 1986 and 1987. Eastern chipmunks and deermice return at equal frequency and time on test and control plots after displacement of about 500 m. Developmental studies on tree swallows indicate similar rates of embryonic abnormalities on test and control plots. However, one test plot has had rates over 35% two years running, and one control plot increased in rate to over 25% in 1987. The rate on all other plots is about 10%. Studies on maximal metabolic rates in winter for deermice and black-capped chickadees indicate no difference for year or plots.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

SUMMARY

The small mammal and nesting bird biological studies for the year 1987 are reported. All previous years' data are also included where 1983-1985 are designated as base-line data and 1986 and 1987 designated as years' when data was collected during low amperage testing of the antenna.

Small mammal community studies showed a lower diversity and species' abundance on the test plot in 1987, however in previous years the test and control plots did not differ. Growth rates of young deermice were not different between test and control plots this year or in 1986, as was the case with age at eye opening and eruption of incisors. The homing abilities of eastern chipmunks and deermice were not different between plots after displacement of approximately 500 meters. Winter maximum metabolic rates of deermice showed no difference between plots or years.

Tree swallow studies focusing on fecundity, survival, growth and parental care found clutch size to be smaller on the test plots in 1987 which was not the case in previous years. Between plots, hatching success showed no difference in 1987, however in previous years the test plots have always had greater success. Egg mortality, and the number of nests failing, was higher on the test plots in 1987 which is consistent with previous years' data. Test and control plots showed no difference in fledging success except in 1985 when the test plots had greater success. The landmark events, age of eye opening and feather eruption, were not different between plots in any year. Growth rates of young tree swallows with respect to body mass, length of the tarsus, ulna and wing were similar for test and control plots for all years.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Egg temperature during incubation showed no difference between plots. Fewer tree swallows returned to the control plot as well taking longer to return the birds homed to their nests on the test plots. Rates of embryonic abnormalities in tree swallows were similar on test and control plots. Winter maximal metabolic rates of black-capped chickadees showed no difference between plots and years.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

PREFACE

This report begins with an extensive statement of the rationale for the studies proposed (see next section, titled "Rationale for Proposed Studies"). Then a section is provided on the overall research design and research facilities. Individual elements of the work are then described in detail in a series of subsequent sections. Each of the sections on individual work elements consists of three parts: (1) a brief restatement of the purpose (rationale) for the work, (2) a detailed description of research methods, and (3) a presentation of representative results gathered during prior years. The presentations of results include discussions of statistical sufficiency, including projections of the sample sizes required to discriminate between test and control plots in future years.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

RATIONALE OF STUDIES

Dozens of species of small birds and mammals are resident near the ELF Communication System, and the operation of the Communication System could in principle affect any of them in any of countless ways. Even with virtually unlimited resources, it would be impossible to monitor individually all ecologically important aspects of all species for possible effects of the Communication System. Accordingly, we have had to exercise informed judgment in selecting variables for study. In this process, we have been guided by two overriding goals.

Our first goal has been to monitor the overall structure of the communities of small animals. Our work in this respect is limited to mammals because the study of the structure of avian communities is the responsibility of another research group. We systematically monitor the species composition, richness, and diversity of the community of small- and medium-sized mammals, and we monitor the relative densities of two major species. By virtue of this broad-scale study of mammalian communities, we are in a position to detect diverse potential effects of the ELF Communication System on the numerous taxonomically diverse species of mammals that are resident near the System. Should the System have any sizable deleterious effects on any one or more species, many of the effects could be expected to affect measures of community richness, diversity, or relative species density, and thus we would be in a position to detect them. This is important in view of the impossibility of monitoring directly all attributes of all species.

Our second major goal has been to focus much of our effort on attributes of individual animals that are particularly likely to be

susceptible to perturbation by the ELF Communication System. The reason for this focus is that laboratory research indicates that if the ELF Communication System is to have effects on birds or mammals, the effects will likely be small, and thus a statistically robust experimental design will be required to detect them (AIBS, 1985). Large numbers of independent measures can be readily obtained on individual attributes, thus facilitating statistical detection of even small effects that the ELF Communication System might have.

In our studies of attributes of individual birds and mammals, we emphasize ecologically significant variables that are especially likely to be susceptible to perturbation. Reproduction and development, for example, receive particular attention because they not only are demographically important but also are more likely to be sensitive to adverse environmental changes than many other animal properties (e.g., Goodposture, 1955; Koskimes, 1950; Kluijver, 1951; Krebs, 1970; Lack, 1954, 1966; Nice, 1954; Perrins, 1965; Perry and Rowlands, 1973). Behavior is studied in depth because it is sometimes modified readily and such modifications can have major repercussions on the lives of individuals and populations (e.g., Cohen et al., 1980; Green, 1979; Morse, 1980; O'Connor, 1978; Slobodkin, 1968).

In the following paragraphs we describe in detail the rationale for each aspect of our work on individual attributes. This work is concentrated on four particularly abundant species. The species have been carefully selected with a view to maximizing their ecological and taxonomic diversity, so as to maximize the probability of detecting whatever diverse effects the ELF Communication System may have. The four are the tree swallow (Tachycineta bicolor), the woodland deer mouse

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

(Peromyscus maniculatus gracilis), the black-capped chickadee (Parus atricapillus) and the eastern chipmunk (Tamias striatus). To facilitate readability in the remainder of the report, they will be referred to simply as the "tree swallow", "deer mouse", "chickadee" and "chipmunk", respectively.

Behavioral Studies

In view of the established sensitivity of certain types of orientational behavior to alteration by the ELF fields (e.g., Graue, 1974; Keeton et al., 1974; Larkin and Sutherland, 1977; Southern, 1969, 1971, 1972a, 1972b, 1973, 1974, 1975, 1976), orientation and homing in the tree swallow, deer mouse, chipmunk, and certain other mammals are being tested to see if they are affected by the ELF Communication System. Specifically, the ability of animals to return to their home-range or territory after displacement is being assessed. We know that animals are able to find food (Krebs, 1970; Royama, 1966) and escape predators (Metzgar, 1967; Watson, 1964) more effectively in their home-range or territory than in less familiar areas. Thus, any disturbance of their ability to return to their home-range or territory after wandering afar could decrease their probability of survival.

The attentive behavior of parental tree swallows and deer mice is being assessed by monitoring visits to the nest containing eggs and young. Disturbance of attentive behavior by the ELF Communication System, if it occurred, could impair development of eggs or nestlings inasmuch as the latter are dependent on parents for both food and warmth (e.g., Balen and Cove, 1972; Hill, 1972b).

Reproduction, Growth, and Development

The frequency and type of prenatal developmental abnormalities are examined in tree swallows (mammals are not studied in this respect because reproductive females would have to be killed to examine fetuses, and such deaths could have serious, adverse effects on population demographics). Prenatal developmental stages are especially likely to be susceptible to perturbation (Axelsson, 1954). There is, at present, no evidence to demonstrate that electric and magnetic fields of the magnitude generated by the ELF Communication System are capable of directly causing embryonic or fetal developmental defects. However, indirect effects are possible. Egg temperatures are extremely important for normal avian development. In particular, eggs must be kept warm by parental incubation. Thus should the incubation behavior of parent birds be disturbed by the ELF Communication System, developing eggs might suffer developmental abnormalities by virtue of experiencing abnormal reductions or fluctuations in temperature. (Zwilling, 1956; Hamilton, 1965).

We monitor aspects of fecundity in both tree swallows and deermice. In the birds, we count the number of eggs produced per female and the number of viable eggs and young per clutch. In the mice we monitor just numbers of young per litter. Fecundity is an important variable to study not only because it is demographically significant but also because it reflects on a number of variables that could, in principle, be affected by the ELF Communication System. Alteration of male or female reproductive physiology could affect fecundity. Further, any serious disturbances of prenatal development

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

in mammals or birds would likely be reflected in a decrease in fecundity inasmuch as abnormal embryos frequently fail to be born (*i.e.*, they are resorbed in utero or fail to hatch) or are eaten or discarded by the parents soon after birth.

Postnatal mortality and the growth and development of nestling tree swallows and deermice are also followed. Any effects that the Communication System might exert on the young themselves could be reflected in altered rates of mortality, growth, or development. Alternatively, disturbances of parental attentive behavior could be influential because the rates of mortality, growth, and development of nestlings are dependent on the extent to which parents provide food and warmth (Hill, 1972b). The size of nestlings at the time of weaning or fledging is of particular interest because when young become independent of their parents, they must become substantially self-sufficient and their maturity can affect their likelihood of survival. Evidence exists that young birds that are of relatively small size at fledging are significantly less likely to survive than ones that grow to larger size while in the nest (Lack, 1966; Murphy, 1978; Perrins 1965).

Maximal Aerobic Metabolism

In the region of the ELF Communication System, low temperatures make winter the most physiologically stressful time of year, at least for animals such as chickadees that live wholly or predominantly above the snow. We study physiological variables that affect the ability of chickadees and small mammals to cope with the severity of the winter climate. Deficits in the physiological ability to cope would be

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

expected to decrease the probability of survival to the next reproductive season.

Birds and mammals keep warm in cold environments by producing heat metabolically to offset heat losses. The extent to which they can keep their body temperature above air temperature depends on how rapidly they can produce heat. In other words, the lowest air temperature at which they can maintain their usual body temperature is a function of their maximal rate of aerobic metabolism (= heat production) (Hart, 1957). In view of these principles, we measure the maximal rate of aerobic metabolism of chickadees and deermice during winter. This peak rate of heat production is informative not only because it determines the lowest air temperature at which thermoregulation is possible but also because it likely provides an index of metabolic endurance. The higher an animal's maximal rate of heat production is, the longer the animal will be able to maintain any particular submaximal rate of heat production (Astrand and Rodahl, 1977; Wickler, 1980). Endurance is important because low air temperatures demanding high heat production can persist for long periods of time.

Beyond its immediate significance for survival in a cold climate, the maximal rate of aerobic metabolism is a valuable variable to measure because it provides an index of physiological health. In fact, peak aerobic metabolism is widely used as such an index in studies of humans. In their classic Textbook of Work Physiology, Astrand and Rodahl (1977) state that "the maximal oxygen uptake is probably the best laboratory measure of a person's physical fitness" if by fitness we mean the capacity of the individual for prolonged heavy work. Brooks and Fahey (1984), in the best of the recent texts on

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

human exercise physiology, state that the maximal aerobic metabolism is "a good measure of fitness for life in contemporary society". Just as peak aerobic metabolism is used as an index of fitness for humans, it can also be so used in studies of animals. A deficit in the peak metabolism among individuals living near the ELF antenna would indicate that some attribute of the all-important systems involved in oxygen supply and use has been adversely affected by the ELF electromagnetic fields. Additional tests would then be required to determine the particular attribute(s) affected. The ability of the respiratory system to provide oxygen, the ability of the circulatory system to transport oxygen and nutrients to metabolically active tissues, the ability of storage tissues (e.g., adipose tissue) to mobilize stored nutrients, and the enzymatic competence of metabolically active tissues to catabolize nutrients are among the variables that influence an animal's peak rate of aerobic metabolism (Wang, 1978). In human studies, peak aerobic metabolism is usually elicited by having individuals run on a treadmill. We elicit peaks by exposing animals to cold, in part because the method is technically simpler than treadmill running (given that animals require extensive training to use a treadmill successfully) and in part because the cold-induced peak is of immediate relevance to understanding winter ecology.

COMMENTS ON AMBIENT MONITORING

We have elected to use weather station data from several nearby sites to monitor the effects of climatic conditions impinging on the plots. The plots are relatively close to each other and therefore experience the same major weather patterns. Minor differences probably

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

exist due to variations in storm tracks, local topography and vegetative features. These differences will produce some degree of variability in response in our study animals, but in most cases we expect this to be small and random in direction. It is therefore our judgment that the greatest value of station weather data will be for examination of year to year effects, rather than within a year among plots.

There is one instance where we have become aware of an effect that is probably based on micro-climatic differences among the plots. The abundance of aerial insects that are preyed upon by tree swallows appears to be greater on test plots, and less affected by cold weather, than on control plots. We have instituted a program to sample aerial prey, in cooperation with Dr. D. Hussell in Ontario, Canada, to examine and hopefully correct for this effect. The program is given in greater detail in the sections dealing with population statistics and growth of tree swallows below.

OVERALL RESEARCH DESIGN AND SUPPORT FACILITIES

To detect possible effects of the ELF Communication System, we compare animal attributes on test plots (test sites) with those on paired, spatially separated control plots (control sites).

Test plots, as specified in the original IITRI Request for Proposals, are areas close enough to the Communication System that electric and magnetic fields attributable to the System, and measured in the soil near the earth's surface, will approximate 0.07 volt/meter and 0.03 Gauss, respectively. Furthermore, electric and magnetic fields attributable to ELF sources other than the System are to be at

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

least an order of magnitude lower than those attributable to the System.

Control plots, according to the original Request for Proposals, are areas sufficiently distant from the Communication System that electric and magnetic fields attributable to the System, measured in the soil near the earth's surface, are at least an order of magnitude, and preferably two orders of magnitude, below those at paired test plots. Furthermore, electric and magnetic fields in the air and earth attributable to ELF sources other than the System (especially 60 Hz sources) are not to differ by more than an order of magnitude between the control plots and their paired test plots.

For purposes of experimental design, the test plot(s) used for any particular work element are paired with particular control plot(s). The plots of a pair are matched as closely as possible for vegetation, soil type, drainage, and other such features. By pairing plots in this way, we minimize the likelihood that non-ELF differences between plots will introduce significant confounding effects into our results.

Different work elements are carried out on different pairs of plots for several reasons. For one thing, certain types of work could interfere with other types if both were carried out on the same populations of animals; areas where we artificially remove animals (e.g., bird embryos), for example, are not used for research on natural populations. Another factor that demands the use of different plot pairs for different work elements is that the various species we study do not all occur in similar habitat types; field habitats are required for the swallows, for example, whereas forests are required for the

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

deermice.

To minimize potentially confounding differences between test and control plots, sham corridors have been cut through the forests at the control plots. These corridors are clearings of the same width as the corridors cut for installation of the Communication System antenna near test plots. They were cut with similar equipment, and they have been treated similarly after cutting. In brief, the sham corridors are as identical as possible to the antenna corridor except that antenna poles and wires have not been installed in the shams. Areas for animal study on control plots and those for animal study on test plots are located about the same distance from the sham corridors and antenna corridor, respectively.

Table 1 summarizes the pairs of test and control plots used for the various work elements, and Figure 1 shows the locations of the plots. The names given to the plots in Table 1 are the standardized ones we use in all our descriptions of experiments and results. Thus, the table should be consulted if uncertainty arises concerning a plot name.

We have established the following standard of statistical sufficiency in our work. In each element of our research, we aim to gather data on a sample size that is at least large enough to give us a 90% certainty of detecting a 20% difference between test and control sites at the 5% level of significance. This is a minimal standard. Where higher standards can be met, they will be. The sample size needed to achieve at least the minimal standard can be projected once the intrinsic variability of the data is known. Research in 1984-1986 has given us information on this variability. For continuous variables, we have used the procedure in Sokal and Rohlf (1981, p. 263)

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

to estimate sample sizes. For discontinuous variables, we have used a Chi-Square procedure described in Gill (1978, p. 82). Table 2 presents necessary sample sizes as currently projected for all elements except tree swallow and deer mouse growth. Discussion of sample size and power of test are presented with the data for each study element (see below).

Our base of operations for the on-site field and laboratory studies is a large house rented in Crystal Falls, MI (801 Crystal Ave.). The physiology laboratory is installed there, as well as the holding facilities for temporary housing of animals used in the physiology experiments. We have a shop for construction and maintenance of field equipment and a large shed for storage of traps, cages, construction materials, and seasonal field equipment. We also have a well established data management system housed there (see below), and living space is provided for employees. We rent and maintain three pick-up trucks to provide transportation between our base of operations and field research sites in all weather conditions on a year-round basis. In addition, we rent a snowmobile and three-wheel all terrain vehicle to gain access to our more remote sites during winter and spring when traveling the entire distance by truck becomes impossible.

For data management we employ an IMS (now LF Technologies) computer system. The system is multi-user and allows storage of data on fixed and removable media. Identical systems are maintained at the field laboratory in Crystal Falls and at the MSU Museum in East Lansing. Data transfer and analysis are accomplished using both systems. Field data are collected by NEC PC-8201A portable computers. We have developed software to standardize and error check field data as it is

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

recorded. Collected data are transferred directly into the LF system at the field laboratory each day. Transferred data are immediately edited and stored on removable and fixed disks for later analysis. Certain data are analyzed as soon as they are collected. This data management design allows us to collect and analyze large amounts of data very efficiently and accurately. In addition, in 1987, we have added high speed tape backup systems to aid in recovery of data should either computer system fail, and for archiving the now voluminous data sets for the various study elements. The large sample sizes required in many of our study elements necessitate the careful and accurate data handling the system provides.

Other major equipment is described in connection with individual work elements in the sections that follow.

Preliminary Measurements on 60 and 76 hz fields

Engineers provided by IITRI have measured 60 Hz electric and magnetic field intensities every year starting in 1983 on our test and control plots, and all the pairs we now use adequately meet the standards for field intensities already described. Electric and magnetic fields produced by the antenna system (76 Hz) were measured starting in 1986 and continuing in 1987, when low amperage testing was begun. We have received the data from the 76 Hz testing this year (November, 1987) and provide a summary of these data. Details of the results of the field-intensity measurements and the measurement techniques can be found in Enk and Gauger, 1983; Brosh, et al., 1984 and 1985; and Gauger, 1987 (personal communication). Earlier discussion of measures and plot pairings are outlined in the 1984 annual report (Beaver, et al. 1985, pp. 3-9).

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

In both years, measures were made in October by IITRI personnel on our test and control plots during antenna testing. In 1986, the antenna was operated from March through October for a total of about 160 hours. The distribution of operation days, start and end times and cumulated time of operation per day for the three legs of the antenna are shown in Figure 2. The active antenna from March to 7 June was the southern east-west leg (EW2); from 17 June through 11 July the northern leg (EW1), and from 22 July through 31 July, the north-south (NS) leg was active. The remainder of the season all legs were activated on a variable schedule (Figure 3). In Figure 3 is shown the time schedules for each of our research tasks during 1986. Testing of the antenna in 1986 involved currents of 4 and 6 amperes for the NS and EW1 legs and 6 and 10 amperes for EW2 leg. About 98% of the on time was with a continuous wave, 76 Hz signal. Schedules of research activities are shown for 1987 in Figure 4, along with antenna on and off times. Testing of the antenna was conducted on a 33% time rotation schedule in which the east-west legs were on together for 5 min, then the north-south leg for 5 min, followed by all legs off for 5 min. The current was 15 amperes, except for 28 April and 22 May when current strength was 3 to 6 amperes. Signal frequency was continuous wave 76 Hz.

Table 1 provides reference to site codes used in tables. Measurement of 60 Hz fields on control and test plots began in 1983. Transverse fields were at or near the lower limits of measurability (Table 30). All values were <0.001 or equaled 0.001 for all sites and plots, except for test plots in 1987. Values for transverse fields were about 21 times higher on test compared to control plots in 1987.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Overall, test and control did not differ significantly (Table 32) but years did due to 1987 test plot values ($F=2.49$, $P=0.04$).

Longitudinal and magnetic 60 Hz fields (Tables 30 and 31) were consistently higher, and significantly different, on test compared to control plots (Table 32). Ratios of the means for test and control plots for each field varied from 1.8 to 5.0 (Table 32). The difference in the strength of these fields varied with the year. Longitudinal fields averaged highest on controls in 1984 and on tests in 1984 and 1985 (year effect $F=4.73$, $P=0.001$). Magnetic fields remained relatively constant on controls but increased during 1986 and 1987 in correlation with low amperage testing of the antenna system (year effect $F=4.0$, $P=0.004$).

The variability in 60 Hz fields within test and control plots (among sites) was also examined by Analysis of Variance. Transverse fields showed no variation (Table 29), but longitudinal fields varied significantly among control sites ($F=8.25$, $P=0.0004$) and test sites ($F=24.61$, $P=0.0001$). Magnetic fields were significantly different for control sites ($F=12.09$, $P=0.0001$) but not test sites ($F=1.82$, $P=0.13$). Year was a significant factor only for test sites for longitudinal ($F=11.65$, $P=0.0001$) and magnetic fields ($F=4.06$, $P=0.005$).

Among control sites, 1C1 and 1C3 (Michigamme North and South) were consistently higher for longitudinal fields. Test sites 1T5 and 1T6 (Ford River North and South) were higher than other test sites in most years (Table 30). Magnetic fields show no patterns in control sites but sites 1T2 - 1T6 all increase a large amount in 1986 and 1987. Site 1T1 shows a smaller increase in these years (Table 31).

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

The control release site (1D3) and Panola Plains plot for tree swallow homing shows small differences in field strength for transverse, longitudinal and magnetic fields (Table 33). However, much larger ratios appear on test release sites (1D1 and 1D2) and their corresponding test sites for transverse and longitudinal fields, with the exception of 1D2 and 1T4 in 1986 (Table 33). It is not clear to us what the significance to attach to the wide annual and site variation observed in these data.

In 1986 and 1987, measurements were made on 76 Hz fields produced by the antenna during low level testing. Control sites were uniform and low (≤ 0.001) for transverse (Table 34) and magnetic (Table 36) fields. Test and control plots did not differ significantly for transverse fields in either 1986 or 1987 (Table 37 - EW had no variation on the controls in 1986 and could not be tested), nor for magnetic fields in 1986. Longitudinal (Table 35) and 1987 magnetic fields (Table 36) were significantly different for test and controls (Table 37), indicating that low amperage testing produced a "treatment" condition on test plots, compared to controls. For this reason, we must consider 1985 as our last pre-operation year.

Variation of 76 Hz fields was examined within a plot (among sites) to see if they were uniform. Control sites were all uniform with respect to transverse fields (Table 34). Sites 1C1 and 1C3 were significantly greater than 1C4 and 1C6 in 1986 and 1987. Among test sites, 1T5 was greater than other sites for transverse fields, and 1T6 was greater than other sites for longitudinal fields. No other patterns emerged. The control sites 1C1 and 1C3 are closer to the

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

antenna system by several Km, perhaps explaining their higher values. It is not clear why the test sites vary.

The release sites for tree swallow homing studies compared to their respective study plots show low ratios for control sites and higher ratios for test (Table 38). Ratios generally increase from 1986 to 1987 corresponding to higher amperage (15 amps) testing of the antenna. This pattern will be examined again below in relation to tree swallow homing results for 1987.

Study of Small Mammal Communities

I. Purpose

The purpose of these studies is to characterize the mammalian communities at test and control sites and to test for possible effects of the ELF Communication System on mammalian community structure. More specifically, the following measures are compared for the two sites and for each site from year to year: species richness (S), diversity (H' , which takes into account both evenness and richness), and species composition (Pielou, 1974). Relative densities of deermice and chipmunks are estimated to test for possible effects of the Communication System at the population level. These studies also provide information on the occurrence of any rare or endangered mammals at the control and test sites.

II. Methods

This year's portion of the study began on 9 August and ended 22 August. Trapping was preceded by a seven day prebait period during which trap doors were locked open. Traps were baited on the first day and then checked and rebaited as needed on the fourth day of the prebait period. The traps were unlocked and rebaited on the seventh day and checked once daily during the following two week trapping period. Longer trap periods such as this increase the chances of capturing trap shy species, and increase the accuracy of relative density estimates used in this study (Smith et al., 1971). Each captured animal was identified to species and marked by toe-clip, furclip or fur dye to discriminate between recaptures and new individuals. Sign surveys were conducted during the prebait and trapping periods to detect the presence of species not likely to be

trapped, such as deer and bear. These surveys entailed searching for and identifying feeding signs, scats, etc., of mammals at each station and between stations. Ten trap stations at 125 m intervals were situated adjacent to both the ELF right-of-way (ROW) at the test site and the sham ROW at the control site with a buffer zone of 75 m at each. One habitat type (mixed deciduous forest) was chosen in order to minimize the effects of macrohabitat differences on community parameters. Each station consisted of six small mammal Leathers live-traps and one raccoon-sized, two chipmunk-sized, and three squirrel-sized Tomahawk live-traps. All traps were positioned in suitable microhabitats within a 15 m radius of each station center. Leathers traps were supplied with polyfil bedding and baited with peanut-butter and rolled oats. Chipmunk-sized traps were set for small carnivores (e.g., weasels) and baited with beef liver or fish. Two squirrel-sized traps were baited with cracked corn (for sciurids) while the third was baited with both fish and liver (primarily for skunks). The raccoon sized trap was baited with carrots, apples, fish and liver. This regime of multiple traps per station helps eliminate bias for species specific preference for certain traps or bait types (Smith et al., 1971). In addition to the ten live-trap stations, two pitfall trap stations were set at each site to capture the smaller shrews (Sorex spp.) which are difficult to live-trap. Each of these stations consisted of ten plastic, 4 quart containers which were set in the ground in a line with approximately 6 m spacing. Each pitfall station was situated midway between two live-trap stations. The positions of these pitfall stations is changed every year. This and the relatively

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

small number of pitfall traps at each site (20) should minimize the effects of kill trapping on shrew populations in successive years.

Species composition, diversity and evenness are calculated from trapping data for species that are trapped, marked and released (we exclude species assessed as present based on sign). The number of animals captured for each species is the sum of all unique individuals trapped over the 14 days of trapping. Species richness is the count of species in the summed 14-day sample, species diversity (H') is calculated as $H' = - \sum p_i \ln p_i$, and evenness is calculated as $H'/\ln(s)$ (following Pielou, 1975), where p_i is the proportion of the abundance of species i in the sample and s is the number of species. The variance of H' is calculated following Hutcheson (1970). The formulation used is a series expansion according to Bowmann et al, (1969), cited in Hutcheson (1970),

$$\begin{aligned} \text{VARh} = & [\sum p_i \ln p_i^2 - (\sum p_i \ln p_i)^2/n + (s-1)]/2n^2 + \\ & (-1 + \sum p_i^{-1} - \sum p_i^{-1} \ln p_i + \sum p_i * \sum p_i \ln p_i)/6n^3 + \dots \end{aligned}$$

where n is the number of all individuals of all species s in the sample.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

A test of the diversity from two samples is also performed following Hutcheson (1970) where

$$t = \frac{H'_1 - H'_2}{(\text{VAR}h_1 + \text{VAR}h_2)^{1/2}}$$

with degrees of freedom

$$\text{D.F.} = [\text{VAR}h_1 + \text{VAR}h_2]^2 / [(\text{VAR}h_1)^2/n_1 + (\text{VAR}h_2)^2/n_2]$$

where n = number of individuals of all species in the sample.

Population densities are assessed using the relative measure described as Trappable Population Number (TPN). TPN values are calculated using the linear regression method commonly used in removal studies (the "Leslie method"; Giles, 1971, pp. 449-450; Smith *et al.*, 1971 and 1975). Removal of trapped animals, however, is not necessary in the present study as individuals are marked when first captured, thus allowing identification of recaptures. Leaving all animals on the plot minimizes the problem of immigration of new individuals because "empty space" is not created.

III. Results - 1987

The adequacy of our sampling effort was demonstrated in prior years following the method suggested by Pielou (1974) (see Beaver, *et al.*, 1985). Total species richness at Michigamme and Pirlot Road sites was 12 and 10, respectively. As in the past, species composition of the two communities is quite similar with most species being common and

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

each community dominated (with respect to number of individuals trapped) by the deer mouse and chipmunk.

The Pirlot community (test plot) had lower diversity (H') than Michigamme, and significantly so (Table 3; $t = 3.045$, $P < 0.005$; t-test due to Hutcheson, 1970, d.f. = 199). In 1985 and 1986, the two communities were not significantly different in H' (Beaver, et al, 1985, 1986). Evenness was higher on Michigamme than Pirlot road (we have no way to test this difference). In past years, the evenness measure has been nearly identical on the two plots. Correlation of frequency of capture at a station by species is significant (Table 3, Spearman rank correlation) indicating a high degree of correspondence between the plots. But the slope of the rank of abundance of species at Michigamme control and Pirlot Road test is not significantly different from zero, although it approaches significance ($t=2.174$, $P=0.073$) (Table 3). In 1985 and 1986 there was a significant relationship between rank abundance on these plots (Beaver, et al, 1986).

In 1987, data from all previous years were re-analyzed as part of a program to standardize and thoroughly error check data bases. Thus, minor changes in TPN and slope figures for earlier years will be found when comparing annual reports from previous years to the current one. The results of the TPN analyses indicated that chipmunk populations were significantly different between plots in all three years (Table 4a, $P < 0.002$, t-test of intercepts, Zar, 1984, p 295). Deer mouse populations were not significantly different on control and test sites in 1985 and 1986, but were significantly different in 1987 (Table 4a, t-test of intercepts). Both species were much lower in abundance in 1987 than in 1985 or 1986. We think the lower numbers in 1987 may have

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

been due to continued impact of Tyzzer's disease. This disease appears when animals are under stress, such as may have been caused by the severe drought in the area in 1986. A number of deermice used in the growth studies were found to be suffering from this disease, and a high percentage of them died. However, populations are known to fluctuate widely from year to year in both species whether the disease is present or not. It is our expectation that between year comparisons will be of little value in assessing ELF effects, and if the results of 1987 continue, between plot comparisons of abundance on plots within years may be only of limited usefulness. It is not clear why plot differences appeared in 1987 and not in earlier years.

Our adequacy of sampling community structure may be examined using the variables with variance estimates; i.e., H' , regression of ranks by plot and TPN. For H' the coefficient of variation is low for test and control plots (C.V. = 1.3% and 0.5%, respectively), which will allow us to detect differences smaller than 5% (Zar, 1984). Our estimates of TPN should also allow us to detect a 20% change, although there are no statistical procedures available to estimate the precise levels of difference we can expect to detect for regression parameters (Zar, 1984).

Data on electromagnetic field strength on test and control plots for small mammal community studies indicate nearly identical values were measured for the transverse and longitudinal electrical fields and the magnetic field Table 4b pre 1986 and 1986. Values in 1987 for 76 Hz fields range from about 8 to 55 times greater on the test compared to the control plot.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

PARENTAL AND NESTLING BEHAVIOR, AND FECUNDITY, GROWTH AND MATURATION STUDIES - TREE SWALLOWS

I. Purpose

The purpose of these studies is to characterize several aspects of the reproductive process in tree swallows at test and control sites and to test for possible effects of the ELF Communication System on these variables. Specifically, the following aspects of the reproductive process are compared between test and control sites and for each site from year to year: parental attentiveness to eggs and young, numbers of eggs per clutch, hatching success within clutches, rates of growth and development of hatchlings, and nestling mortality. All of these work elements are described together in this one section because they are all carried out on the same populations of birds.

II. Methods

These studies are carried out in natural or artificial clearings where we have erected arrays of nest boxes. The boxes are made of cedar lumber and are mounted on posts, 1.5 m above the ground. Tree swallows readily elect to nest in the boxes and will tolerate considerable disturbance by investigators. The boxes can be opened to permit inspection and weighing of young. Sheets of high-density polyethylene wrapped around the posts prevent access by terrestrial predators.

When possible, adults are captured on the nest after incubation is completed and banded with U. S. Fish and Wildlife Service bands for identification. Since it has been shown that certain reproductive variables are affected by the age of the female (DeSteven 1978), most

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

of our effort is placed on capturing females. In addition, as many young as possible are banded before fledging.

Active nests are checked daily or every other day to determine the dates that eggs are laid, how many are laid, the dates the young hatch, and overall hatching success. Monitoring of the nests for nestling growth and mortality then continues until all young have reached 16 days of age. Young tend to fledge unusually early if disturbed beyond day 16. Therefore, after day 16, nest checks are done every other day with minimal disturbance to estimate fledging success.

For studies of egg incubation, temperature-monitoring equipment is used. The tip of an EME Systems thermosensitive probe is inserted within a simulated egg, and the simulated egg is placed among the natural eggs of the clutch. The thermosensitive probe then signals when the clutch is being warmed by the parent. Data from the probe are recorded every 3 minutes, 24 hours per day, using On-site Weather Loggers, made by EME Systems, and NEC microcomputers. Simultaneously, the air temperature outside the nest box is monitored and recorded using a second probe.

Parental attentiveness to nestlings was monitored using video recording equipment in 1986. However, our analyses showed these data to be too variable to meet statistical sufficiency. We therefore have dropped this procedure from our studies.

For studies of growth and development, nestlings are weighed every other day with a Pesola spring scale accurate to 0.1 gm. The lengths of the tarsus, ulna, and wing (all from the right side of the body) are measured with dial calipers accurate to 0.1 mm.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Since it is impossible for one observer to measure all nestlings we have at least two observers collecting growth data. However, we've noticed that different observers differ slightly in their techniques for measuring weights and body parts therefore, we have all observers rotate among the plots so that every nestling is eventually measured by all observers. Regularly rotating the observers in this way has the effect of submerging the variance in measurement, due to observers, into the error in each nestling's growth curve. This measurement protocol unfortunately prevents us from being able to block observer effects in the statistical design. However, as we show below, when we use data from each individual bird's growth curve, even the significant effects of differences in observer technique do not prevent us from being able to detect very small differences in patterns of growth.

For analysis of growth data, we use the procedure for fitting growth data to models of growth proposed by Ricklefs (1967) and used previously for tree swallows by Zach and Mayoh (1982). Briefly, the data for each nestling are subjected to curve fitting using an exponential or logistic model in a regression routine in SAS (Statistical Analysis System). The model of best fit, as judged by having the highest value of R^2 , is used in subsequent analyses to obtain the rate of growth, the intercept, and the inflection point. The model of best fit every year, including 1987, has been the logistic.

In past years we have detected significant differences in growth rates of young tree swallows between test and control plots. Recent evidence suggests that food availability on a plot can have a significant effect on both clutch sizes and growth rates of tree

swallows (Hussell and Quinney, in press; Quinney et al., 1986). In order to determine what degree of variation between test and control plots in growth rates is the result of food resource availability, we have undertaken steps to quantify the flying insect abundance at each site. We have communicated with Dr. Hussell of the Ontario Ministry of Natural Resources and have designed a sampling scheme based on his earlier work (see Hussell and Quinney, in press, for detailed methodology). At each tree swallow site we collect flying insects during the daylight hours in two suspended conical nets with alcohol traps. These nets are located among the nest boxes and are constructed to face passively in the wind so as to continually sample insects which either fly or are blown into the nets. Previous studies show an excellent relationship between the insects collected in this type of system and the insects delivered to young swallows in the nest by their parents (Quinney and Ankney 1985). Sampling begins before the initiation of any egg laying and ends when all young from the plot have fledged. After insects are sorted into size classes we compute an index of the biomass of flying insects determined from daily catches on each plot. This will allow us to compare the prey abundances between test and control plots and help explain differences in growth rates between plots not due to age of the adults or clutch sizes. These data will further refine our abilities to detect possible subtle differences in tree swallow reproductive measures due to electromagnetic fields associated with the Communication System.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

III. Results - 1987

Tree swallow plot names, numbers of boxes at each plot, and percent occupancy for 1985-87 are shown in Table 5. Small differences in number of boxes on some plots will be noted when compared to earlier annual reports due to attrition or addition of boxes. With the placement of additional boxes on plots in the early spring we now have a full complement of bird boxes at test and control sites. Of the 308 nest boxes monitored in 1987, 232 (75%) had egg-laying activity which is a continuation of the increase in activity observed between 1985 and 1986. This increase is due, in part, to the additional opening caused by completed cutting of the sham corridors around the perimeter of some of the control plots, the roller-chopping of encroaching aspen by the Michigan Department of Natural Resources, and by our efforts at predator-proofing of nest boxes. In early spring, all of the nest box poles were wrapped with a high density polyethylene to help prevent access by terrestrial predators. With increased return rates of nesting adults observed each year we have established plots which will provide adequate sample sizes for all of the tasks reported on below. Starting in 1986, we conducted all aspects of the research program on specific plots established for each individual task (see Table 1) and will continue with this protocol as originally proposed.

The age of adults breeding on the plots was quantified in earlier years by categorizing a bird as an adult if it had a high percentage of its back plumage colored iridescent green. Younger birds have mostly a gray back plumage with little green (DeSteven 1978). In 1985, we found many more young birds nesting on control than test plots (Beaver, et al, 1986). In 1986, we were not able to make as complete a

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

determination because many birds abandoned their nests due to inclement weather prior to the time we designated to assess age of adults. However, we did keep records of birds we saw on our daily visits to the plots. Less than 10% of nesting birds were young birds and there appeared to be equal numbers of them on test and control. In 1987, less than 20% of nesting birds were young birds, with greater numbers of young birds on the control plots.

Summarized fecundity data for tree swallows in 1987 and comparisons to 1985 and 1986 are presented in Table 6. These data were collected from the Pirlot Road test plot and Tachycineta Meadows control plot and exclude any renesting attempts. Mean clutch size 1987 at Pirlot Road (5.0 eggs/nest) was similar to Tachycineta Meadows (5.2 eggs/nest; $t = 0.938$, $P > 0.3$). Both of these values are within the range of those reported elsewhere for tree swallows (Chapman 1955, DeSteven 1978, Zach and Mayoh 1982). Until 1987, Pirlot Road test plot has had consistently higher clutch sizes in previous years. We have suspected there are differences in available food at the test and the control plots and this could be influencing clutch size, a finding reported for tree swallows in Canada by Hussell and Quinney (1987). As reported in 1986 we are cooperating with Hussell in determining prey biomass at our sites and we should be able to examine this using the data we have on insect biomass as soon as the analysis of our insect data by Hussell is complete. There was no difference in the distribution of clutch sizes between test and control plots during 1987 or in prior years (Table 6, G tests of independence).

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Hatching success (Table 7) was greater at the Pirlot Road test plot (85.1%) than at Tachycineta Meadows (83.8%) during 1987 and this difference in likelihood to hatch is non-significant (G test of independence, Sokal and Rohlf 1981, $G = 0.067$, $df = 1$, $P > 0.5$). When 1987, 1986 and 1985 data are analyzed together, likelihood to hatch is shown to be independent of both plot and year ($G = 5.89$, $df = 5$, $P > 0.25$). The actual number of young which hatched per nest (Table 6) was the same in 1987 for test and control (4.2 young/nest), this value being within the range reported elsewhere (Low 1934, Paynter 1954).

Fledging success was slightly lower at Pirlot Road (72.1%) than at Tachycineta Meadows (75.8%) in 1987 (Table 8), but this small difference in likelihood to fledge is not significant (G test of independence, $G = 0.3$, $df = 1$, $P > 0.5$). When 1987, 1986 and 1985 data are analyzed together (Table 8), likelihood to fledge is highly dependent upon year and plot location ($G = 115.78$, $df = 5$, $P < 0.001$). When this 8 X 2 table is broken down into its components of year (three years pooled over test and control) and plot (control and test pooled over years), there are no detectable year effects ($G = 1.15$, $df = 2$, $P > 0.5$) or plot effects ($G = 0.01$, $df = 2$, $P > 0.9$). However, plot and year are not independent of one another ($G = 22.3$, $df = 2$, $P < 0.001$) and there is significant interaction between them ($G = 114.6$, $df = 8$, $P < 0.001$). So, if each year is tested separately (likelihood to fledge on test vs. control for each year), we find no significant departure from independence, except in 1985 when young were much less likely to fledge on the control than on the test ($G = 5.82$, $df = 1$, $P < 0.025$). As noted in previous annual reports, 1985 was the first data collection

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

year on the control plots and significant numbers of inexperienced breeders were nesting there. It is well documented in the literature that first year tree swallows are less successful than their older counterparts (DeSteven 1978). The actual number of young to fledge per nest during 1987 (Table 6) was the same at the Pirlot Road test plot as at Tachycineta Meadows control (3.1 young/nest).

When data on clutch size from the last three years are considered together (Table 8a), we observe a significant effect due to plot ($F = 4.2$, $P < 0.05$); the clutch sizes on the test plot being larger in both 1985 and 1986. This trend is reversed in 1987, resulting in a significant interaction of plot and year ($F = 3.14$, $P < 0.05$). No overall effect of year was detected. Once again, we hope to elucidate the reasons for these differences using the aerial insect prey biomass data which are presently being worked up by Hussell in Canada.

When hatch rate data from the last three years are considered together (Table 8b), we find no significant effects due to plot, year, or plot/year interaction (all $P > 0.14$). This is interesting in light of the clutch size differences observed some years; approximately equal numbers of young hatch in each nest even though there are differences observed in clutch size.

When data on actual numbers of young fledged per nest from the last three years are considered together (Table 8c), we detect no significant effects due to plot or plot/year interaction, yet there is a highly significant effect of year ($F = 11.02$, $P < 0.001$). This effect is primarily due to the episode of inclement weather in 1986 which severely limited the numbers of young fledged from most nests.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Just over one young/nest fledged in 1986, compared to 2.6/nest in 1985 and 3.1/nest in 1987.

The landmark events of eye opening and primary feather eruption are presented in Table 9a. Mean number of days to eye opening in 1987 was longer at the Pirlot Road test plot (7.4 days) than at Tachycineta Meadows control (6.7 days), however these differences were not significant (Table 9b, $P > 0.30$). Eyes opened much earlier in 1986 on both the test and control when compared to 1987 (Table 9b). The scoring in the field of eyes closed or open is somewhat subjective and may be biased depending upon observer, lighting conditions and other factors. In addition, we only observe the young on an every-other-day basis. The resulting increase in the variance further reduces our ability to detect subtle differences in age of eye opening. We will continue to score the age of eye opening, but with increased attention to problems in assessing the status of the eye.

Mean number of days to feather eruption in 1987 was the same at both test and control (8.5 days). Analysis of the 1987 data show no significant effects of plot (Table 9c, $P > 0.94$). Contrasting feather eruption with eye opening, the eruption of primary feathers is generally a less variable measure than eye opening and is much less subjective in the field when the actual scoring takes place. We therefore have more confidence in using this variable as an assessment of ELF effects on developmental landmarks.

Exposure data for nests, eggs, and nestlings used to assess mortality rates were calculated using the Mayfield method (Mayfield 1961, 1975) and are presented in Table 10. Units of exposure are egg

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

days, nestling days, and nest days. For example, one nest with five eggs observed for four days would represent 20 egg days and four nest days of exposure. Data presented here include all active nests from all plots and represent an overall nesting success analysis.

Egg mortality was significantly higher on the test plots (G test of independence, $G = 24.324$, $df = 1$, $P < 0.001$), while nestling mortality was not significantly different between the plots ($G = 0.779$, $P > 0.25$). Overall nest mortality (e.g. failure of an entire nest) was significantly different between pooled test and control plots ($G = 7.723$, $P < 0.01$), and there was a significant difference between test and control plots when nest mortality is partitioned between the incubation phase but not the nestling phase ($G = 7.642$, $P < 0.01$ and 0.068 , $P > 0.75$, respectively). This is the first year we have detected consistent and significant differences in mortality of eggs and nests between test and control plots. The fact that mortality is proportionally higher on test plots this year coincident with low power (amperage) testing of the antenna is suggestive of an effect, but we must await further data from next year and beyond when the antenna is scheduled to be fully operational before conclusion of any effect is warranted.

In 1987, 210 adults were captured; 184 (87.6%) were new individuals and 26 were returns (12.3%) which were banded by us during previous seasons. The number of returning adults this year was less than previous years when 29.7% returned in 1986 and 16.6% returned in 1985. In addition, as many young as possible are banded before fledging; in 1987, 609 young were banded in the nest. The number of young banded is approximately a twofold increase over previous years'

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

efforts, primarily because more nestlings lived through the season this year. The high mortality of young in 1986 caused by inclement weather significantly reduced the number of young available for banding.

Curve fitting to growth data for individual birds during 1987 for body mass, tarsus and ulna growth was accomplished using the logistic model while wing growth was fit by the exponential model. These models produce the highest R^2 values, on average, compared to other growth models (see Zach and Mayoh, 1982, for discussion of various models).

The logistic model was then used to produce values for rate of growth at the inflection point and the inflection point for use in an analysis of variance. The growth and inflection point variables for each nestling were included in the data set if there was a significant correlation between the variable and age. The data were then analyzed using nested analysis of variance (NANOVA), with the effect of nests included within plots. Thus, the model may be written as:

$$Y_{ijk} = u + a_i + B_{ij} + e_{ijk}$$

where Y_{ijk} is the k th observation in the j th subgroup of the i th group, u is the parametric mean of the population, a_i is the fixed effect of the i th group (plots), B_j is the random contribution of the j th subgroup (nests) and e_{ijk} is the error term. A nested model was used to account for the known effect of parents on the growth of their nestlings. Ricklefs and Peters (1981) studying the European starling (Sturnus vulgaris) in Pennsylvania found the most significant contribution of variance to total variance in growth was due to the parents rather than variation in individual nestling growth or inherited growth traits. Our data on tree swallows shows similar

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

partitioning of the variance in growth. The appropriate ratio for testing for a treatment (plot) effect is the mean square due to plot with the mean square due to nests within plots rather than the error mean square. This reduces the effective sample N to the number of nests, rather than the number of nestlings, and has some important impacts on the power of the test. This will be discussed in detail below after summarizing the findings for 1987.

In general, growth rates and inflection points (the intercept was not used in the analysis because its meaning from a biological point of view is not clear) were most strongly affected by nests within plots and least by plot (Tables 11a - 14). For weight, tarsus, ulna and wing growth constants, no significant plot effects were detected in 1987 or in previous years. For weight, tarsus and ulna inflection points (wing growth model does not have an inflection point), there was also no plot effect in 1987 or previous years. (The reader should be informed that in previous reports, we have noted some significant plot effects. These effects have been found to be due to errors in data files, inclusion of individuals with either clearly abnormal growth or with very few measured points. We instituted procedures for trapping such errors in 1987 and have applied these techniques to all previous data bases in the re-analysis shown in this report. Because of this, the numbers in some of the tables will not match those in previous annual reports.) However, for all variables, except for ulna growth in 1987 and 1986 (Table 13a), a highly significant effect was found for nests within plots. Thus, nests differ greatly between themselves, but not between plots, for the measured variables. We do not currently understand why nests showed no significant variation in ulna growth in

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

1986 and 1987 but did so in 1985. Tables 15a 15b present the means and standard statistics for each variable.

We have examined the power of each performed test and the difference in means that can be detected with our current data (Zar, 1984, p 260). The results (Tables 16a, 16b) indicate that we are able to detect differences in test versus control means of less than 10% in most variables for growth and inflection point, which is half of our stated detectable difference. The main exception is for tarsus growth in 1986 and for tarsus inflection point in all years. However, the power of the performed tests does not meet our criterion of 90% in any year for any variable. The power of a test varies with the sample n, the difference in means one wishes to detect and the variability of the data. Of these variables, only the detectable difference can be adjusted for data already collected. In Tables 16a and 16b, we provide power of the test for means which are scaled to be exactly 20% different and then we recompute the power of the test. Power is dramatically improved for all variables except tarsus growth and inflection point. In general, 1985 yields the lowest values for power and the value improves in 1986 and 1987. We feel this reflects improvement in measurement technique and quality control of data collection. However, this analysis clearly points to problems with the measurement of the tarsus. Examination of raw data on tarsus growth shows there is more variation in the measured length on the same individual from day to day than for other variables. It appears that individual field workers are having a significant impact on the data for tarsus, and therefore we must re-evaluate our procedures.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

The analysis of power and detectable difference allows a more detailed examination of the method of analysis we are using for tree swallow growth. One striking feature if the growth data fitted to the logistic model (or any of the other growth models) is that the coefficient of variation is higher, by about 10%, for all variables compared to the raw data itself. Thus, the fitting procedure is introducing additional, undesirable variation into the data. We are now investigating other statistical procedures that are able to use the raw data directly, such as Repeated Measures ANOVA. This procedure may perform better than fitted parameters in examining growth of nestlings.

Data on electromagnetic fields on the plots used for the studies of clutch size, hatching success, growth, landmark developmental patterns and fledging success indicate very similar and low levels for pre-1986, 1986 and 1987 periods (Table 4b). Thus, the electromagnetic environment does not appear to have changed as much as between other plot pairs from the pre- to post- antenna testing phase.

Incubation - During 1987 a total of 19 nests were studied (8 test, 11 control). The variables considered were the temperature of the eggs during incubation as well as the corresponding ambient temperatures recorded at each nest. Daily means for ambient and egg temperatures at each nest were used in a nested analysis of variance (NANOVA) to detect any differences between plots. In addition, the ambient temperature was used a covariate in the analyses to correct for any differences in the thermal environment of the nests within plots as well as any overall differences between plots.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Incubation does not begin abruptly in the tree swallow. Rather, the variance of the incubation temperature decreases and the mean incubation temperature increases over the course of the first four days of incubation following the laying of the last egg. Similar patterns have been shown in other passerines as well (Haftorn 1987; Prescott 1964; Skutch 1962, 1976; Zerba and Morton 1983a, 1983b).

The NANOVA (Table 17a) shows there is no significant difference ($P > .5$) between plots in the temperature of the eggs during the course of incubation. Also, there is no significant effect of nest within plot ($P > .07$), although this relationship is approaching significance. This indicates that much of the variation observed is at the level of the nest. This could be caused by several factors, including the female exhibiting highly variable incubation strategies, and the placement of the probe in the nest in relation to the other eggs. We took steps to reduce the impact of probe placement beginning in 1987. The position of the probe, the position of the eggs, or the shape of the nest can all be manipulated at the initiation of data collection, for optimal placement and generally, no further changes beyond the first day are needed.

When the ambient temperature is used as a covariate (Table 17b), once again there is no differences between plots in incubation egg temperatures ($P > .9$). There is however, a significant effect due the the ambient temperature ($P < .0001$) and a significant effect of nest within plots ($P < .03$). In other words, the amount of variation shown attributable to plots is decreased where the ambient temperature is taken into consideration.

Electromagnetic fields were lower on Panola Plains control than

North Turner Test (Table 4b), especially in 1987. The significance of these differences to incubation are not yet apparent.

PARENTAL AND NESTLING BEHAVIOR, AND FECUNDITY,
GROWTH, AND MATURATION STUDIES - DEERMICE

I. Purpose

The purpose of these studies is to characterize several aspects of the reproductive process in deermice at test and control sites and to test for possible effects of the ELF Communication System on these variables. Specifically, the following aspects of the reproductive process are compared between test and control sites and for each site from year to year: maternal attentiveness to nestlings, numbers of young born per litter, proportions of young surviving until weaning, and rates of growth and development of nestlings. All of these work elements are described together in this one section because they are all carried out on the same families of mice.

II. Methods

These studies are carried out within enclosures because free-ranging mice have been found not to remain resident in nest boxes for long enough periods for us to obtain the data desired. The enclosures are large: 6.1 by 5.8 m. Ten enclosures have been constructed within mixed deciduous forests at both the test and control plots. They are open at the top to allow free passage of atmospheric electromagnetic fields and free exposure to weather. Furthermore, they are constructed mostly of acrylic plastic sheeting, which is permeable to atmospheric electric fields according to IITRI engineers. Briefly, the walls of

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

the enclosures consist of acrylic sheeting attached to cedar posts; the walls project about 15 cm below ground to prevent mice from digging out, and they extend about 60 cm above ground. A 51-cm-wide sheet of acrylic is placed horizontally along the top of each wall to prevent animals from climbing over the wall. Tree trunks are sheathed with sheets of high-density polyethylene to prevent mice from climbing in or out of the enclosures via the trees. Each enclosure is provided with a nest box and a feeding and watering station. The nest box can be opened to permit access to the mice.

Small enclosures (termed holding facilities) built according to the same design, but measuring just 1.2 by 1.2 m, are also constructed at the same sites. These enclosures are used as holding facilities for mice awaiting study in the large enclosures.

The mice to be studied are captured in mixed deciduous forest near the enclosure sites. They are set up as male-female pairs. Then later the females are transferred into the large enclosures when visibly pregnant. They give birth in the enclosures and rear their young to the age of weaning.

The attentive behavior of the mother mice toward their young is monitored using treadles attached to the nest boxes. A treadle is also placed at the feeding station to monitor time spent there by the female. Treadles follow the design of Hill (1972b) and Dice (1961). Each is enclosed in a tunnel, which is positioned over the entry into the nest box or feeding station so that the mother must pass over the treadle to enter or exit. Movements of the treadle activate a mercury switch whose signals are processed in an A/D device (EME Systems). Signals from the A/D device are recorded continuously on a NEC

microcomputer, 24 hours per day. From the records, it is possible to deduce the time of each entry and exit, and thus it is possible also to compute the durations of periods spent in and out of the nest box. Because a treadle system of this sort can monitor the movements of only a single animal, the male parent cannot be present, and monitoring of the female can be carried out only until the young are about 16 days old (for at that age the young themselves start to exit and re-enter the nest box).

Newborn young are toe-clipped for identification when 4 days old. From then until they are 22 days old, their growth is followed by weighing every other day to an accuracy of 0.1 g using a Pesola scale. Initial litter size and subsequent deaths are recorded. The age of eye-opening is recorded as an index of developmental rate.

III. Results - 1987

The growth and development of 9 litters from 9 females at Pirlot test plot and 7 litters from 7 females at Michigamme control plot were monitored during 1987. All litters used in the analysis were born before July 12 as the deermice fail to exhibit a substantial late summer reproductive peak typical of deermice in this region (Baker, 1983).

Summary statistics of growth studies are presented in Table 18a. A perusal of the growth in body mass of nestlings indicates that growth curves often appear non-linear. Although littermates consistently exhibit similarly shaped growth curves, there are apparent differences in curves among litters of different females as well as differences between litters of the same female (i.e., some are exponential, some

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

sigmoidal, etc.). While this variability in the shape of growth curves among (but not within litters) is interesting, it precludes the use of any particular non-linear model (e.g., logistic growth model) to estimate and compare growth rates in these mice. Therefore, growth rates have been estimated using linear regression analyses for growth of each individual up to the time of weight recession which appears to be correlated with weaning. A linear regression of combined growth of all individuals of each litter was also performed. For the sake of clarity, only the latter will be presented here. Nested ANOVA of growth rate due to mothers nested with plot yields a significant effect of mother but none due to plot (Table 18b). At this writing, we do not have any hypotheses as to the nature of the mother effect.

The power of the test and the detectable differences are estimated for 1986 and 1987 data (Table 19). The minimum detectable difference decreased from about 25% in 1986 to about 15% in 1987. However, the power of the test changed from about .32 to less than .30 from 1986 to 1987. These results correspond with our findings for detectability and power in tree swallow growth, and perhaps reflect the same problems in field measurement and inherent variability in the data.

Age at eye opening and incisor eruption in deermice are similar between plots (Table 20a). Age at eye opening was not significantly different between plots in 1987 (Table 20b) as was the case with incisor eruption (Table 20c). Much of the variation can be attributed to the frequency of visits we can make to obtain the data (now at every other day). Thus an animal categorized as not having eyes open on a particular day will not be checked again for two days. This produces a built in error of two days. Thus, we do not feel we can obtain fine

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

enough resolution for these variables to meet our statistical criteria without increasing the frequency of visits. We are investigating this possibility within our present work schedules.

Electromagnetic field strengths were lower on Michigamme North control plot compared to Pirlot Road test plot (Table 4b), especially in 1987.

HOMING STUDIES - TREE SWALLOWS

I. Purpose

The purpose of these studies is to measure the homing success of tree swallows at test and control sites and to test for possible effects of the ELF Communication System on such success. Variables measured are the proportions of swallows that successfully return home after displacement and the time required for each bird to return home.

II. Methods

Adult birds are captured at the nest box using a passive nest box trapping device (Hussell, per comm). Captures take place between 0930 and 1230 to allow adequate feeding of the young in the nest prior to capture. Following capture, each bird is sexed (using the presence of a cloacal protuberance for males and brood patch for females) and aged using plumage characteristics (Hussell, 1983). Birds are banded using a standard U.S. Fish and Wildlife band and are color marked on the breast using "magic markers" to provide rapid and positive identification while in flight. Birds are placed in wire cages which are covered with black cloths, and then driven to the release sites.

In our first studies of swallow homing in 1984 and 1985, we

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

released birds at all four cardinal compass directions (east, west, north, south) at test and control sites. The results revealed no differences in homing success from one compass direction to another. Furthermore, because tree swallows probably home without regard to habitats they fly over, and they are not likely exposed to any different hazards (predators, etc.) in homing from one direction as opposed to another, we feel justified in releasing birds at just one compass direction. Using just a single release point at test and control sites is more efficient in terms of personnel effort than use of four release points and thus permits adequate sample sizes to be obtained more expeditiously.

The release points are located in open areas that are at a distance of 30 km from the nest sites and at a compass direction 20 degrees NE of the nest sites (see Figure 1). This value of 30 km was chosen because it is greater than the distance corresponding to a drop of two orders of magnitude of potential electromagnetic fields given off by the Communications System. The direction of the release points in relation to the nest sites was chosen so that birds attempting to return to the test site in a straight line will cross both east-west legs of the antenna configuration -- areas that would supposedly be maximally influenced by ELF electromagnetic fields. Upon release, the time, vanishing vector, and weather conditions are noted. Observers located near the nest boxes record the time at which the birds return.

III. Results - 1987

The numbers of birds used for homing and the likelihood to return are presented in Table 21a. These data are from the Panola Plains

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

control plot and pooled from Cleveland Homestead and North Turner test plots. Test plot data were pooled since there were no significant differences in return rates due to test plot location. Likelihood to return to the nest site on the test plots was 97% (36 returned of 37 displaced) and 66% (25 of 38) on the control plots; these differences were significant ($\chi^2 = 12.259$, $df = 1$, $P < 0.001$). Mean time to return (Table 21b) was lower on the test plots (155.1 min.) than on the control (202.4 min.), and these differences were also significant (t-test, $t = 3.6$, $P < 0.001$). All of the young in the nests of birds that returned were reported in healthy condition. In summary, the birds displaced from the test plots had a much higher likelihood to return as well as faster return rates when compared to the controls.

When return times from 1986 and 1987 are considered together (Table 21c) no significant effects of year or plot/year interaction were detected, yet there was a highly significant plot effect ($F = 12.29$, $P < 0.0007$). This plot effect is primarily due to an accentuation of the effect observed in 1986. Table 21b shows that return times on the test plots have remained essentially the same over both years, whereas on the control plot the times increased. The difference between test and control return times in 1986 was approximately 27 minutes which increased to 47 minutes in 1987. The reasons for this increase coupled with the concomitant increase in the likelihood to return are very interesting, yet unexplained at this time. We have further classified the birds used in the homing study as to gender and age (Table 22). Gender is very nearly balanced, whereas age is less so. Statistically, the subdivision of the data reduces the sample size, which results in non-significant effects for differences in homing. We may be able to

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

pool data over years and increase our sample size in each category. This may then let us assess the effects, if any, of gender and age on homing.

However, we must be concerned about the possible relationship of homing performance and antenna testing, which began in 1986 and continued in 1987 with increased amperage and frequency of operation (Tables 34-36). We can find no unusual patterns of 76Hz or 60 Hz EM fields on the control capture plot (1C4) and the release site (1D3), except for their very much lower values for most EM fields compared to test capture and release sites and that the ratios of test release and capture sites are much higher (Table 37). It is therefore, not clear what is causing the pattern between control and test plots. We propose to have measures of EM fields made along the routes of flight from the release sites to the plots at intervals of 5 Km in 1988 to see if anomalies exist that could be disrupting birds attempting to home to control plots. Maps indicate there are major 60 Hz distribution lines and generating facilities directly between the control release and capture site.

HOMING STUDIES- SMALL MAMMALS

I. Purpose

The purpose of these studies is to measure the homing success of small mammals at test and control sites and to test for possible effects of the ELF Communication System on such success. Variables measured are the proportions of individuals that successfully return home after displacement and the time required for each individual to

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

return home. The principal species studied are deermice and chipmunks.

II. Methods

The small mammal homing study is conducted on two trapping grids, one at the Pirlot road test site and the other at the Michigamme control site. Each grid contains 100 stations spaced ten meters apart, with one Leathers live-trap placed at each station baited with peanut butter and rolled oats. The grids were situated on the east side of both the ELF ROW and the sham ROW. A habitat buffer between each ROW and its respective trapping grid was increased this year to 50 meters, rather than the 10 meters of 1985. This increase helped insure that both the grids and their displacement lines were located in more uniform habitat, one of continuous mixed deciduous forest dominated by sugar maple (Acer saccharum).

Trapping began on 4 July and ended on 3 August, 1987. Traps were checked twice daily (ca. 0800 and 1700) and rebaited as necessary. Because of the small sample sizes obtained for other species in 1985, only eastern chipmunks and woodland deer mice were displaced this year. Each animal was weighed, sexed, and toe-clipped or ear-tagged for individual identification. Reproductive condition, station number, and capture time were also recorded. Individuals were kept for displacement after their third capture; such animals were deemed to be residents of the area where the trapping grid is established which, hopefully, insured their detection by continued recapture on the trapping grid upon returning from displacement. Before being displaced, each animal was kept in a laboratory cage supplied with

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

nesting material, lab chow, and water. Cages were placed in screened-in storage sheds located near each site. Displacements took place during, or just prior to, the next activity period following capture; deermice (nocturnal) were displaced at dusk (ca. 1900) and chipmunks (diurnal) were displaced in the morning (ca. 0800). Each animal was displaced 450 m from the trap it was captured at when kept for displacement. Displacements take place to the south and west of the home grids. The exact point of release is adjusted to reflect the point of capture on the home grid; this way all individuals are displaced exactly the same distance from their capture point. Trapping continued for five days after the last animal was displaced.

During our initial studies on mammal homing in 1985 (Beaver, et al., 1986), we displaced chipmunks and deermice in all four cardinal directions in order to investigate any directional biases in homing ability. No such biases were found even though animals displaced west and north on the control and test plots had to cross the sham corridor or actual antenna corridor, as well as somewhat different habitat types. However, our sample sizes were small for any particular displacement direction (maximum of 10 animals) and we therefore could not be certain of the robustness of our tests. Thus, in contrast to the work on swallow homing, we decided to reduce the number of displacement directions to two rather than one. Reducing the number of directions from four to two increases efficiency of sampling. By using two directions rather than one, however, we maintain the diversity of habitats and corridor crossings at each site, thus helping to insure that we are further able to examine the effects of habitat conditions as well as potential effects of ELF on homing behavior.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Displacements take place to the south and west of the home grids. The exact point of release of a displaced animal is adjusted in relation to the point of capture on the home grid so that all individuals are displaced the same distance from their area of capture on the home grid. The home grids and the release areas are located within relatively continuous northern hardwood habitat. Once an animal has been displaced, traps on the home grid are checked morning and evening for at least 5 days. In this way, we monitor the numbers of animals that successfully return, and we can compute the minimum amount of time required to return within approximately 12 hours.

The displacements to the south are through continuous forest, whereas those to the west require returning animals to cross the antenna corridor at the test site and the sham corridor at the control site. Use of the two displacement directions thus specifically allows us to test for directional differences in return rates which might occur due to the fact that animals returning from the west must pass beneath the antenna line -- potentially the area of greatest electromagnetic disturbance.

III. Results - 1987

The number of animals captured in 1987 was similar to 1986, however deermice were trapped more often than chipmunks which was opposite 1986 results. Overall, population numbers remain very low at both test and control sites. The assumed reason was the presence of Tyzzer's disease in wild populations of deermice and chipmunks. We reported earlier that trappable populations of these species were down from 1985 and 1986 for the presumed same reason.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

A total of 32 deer mice (9 control, 23 test) and 16 chipmunks (4 control, 12 test) were displaced (Table 23). Likelihood to return to the home area was assessed using a G-test of independence (Sokal and Rohlf 1981, p. 737). Due to the low sample size differences in returns with respect to sex or displacement direction could not be assessed. No difference was detected in the likelihood to return for chipmunks between the test and control sites ($G = 0.3034$, $P > 0.50$) or deermice ($G = 0.0234$, $P > 0.75$). Return rates of chipmunks for 1986 and 1985 were significantly different (adjusted $G = 7.689$, $P < 0.01$) with a higher proportion of the displaced individuals returning this year than last. This was to be expected, however, since the displacement distance for chipmunks was reduced from 1985 (500 m to 450 m). Differences in likelihood to return between years for deermice was not assessed due to the large disparity in the number of individuals displaced each year (32 in 1987, 9 in 1986, 71 in 1985). Generally, deermice are the most abundant small mammal on our forest study sites and hopefully, population numbers will increase to former normal levels. Winter trapping during 1985-1986 showed that population numbers had declined drastically since summer 1985 and these low numbers persisted throughout summer 1986, as shown by the small numbers of displacements during the homing study and the low TPN estimates during the community study. This continuing trend was confirmed during the winter trapping in 1987 as well as the 1987 summer field work. In past years, deermice have been the most abundant small mammal on our forest study sites. We have taken steps to ensure adequate numbers of individuals to home in the future by increasing the size of our homing

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

trapping grids from 0.8 ha to 1.8 ha. Resetting the grids was done at the completion of the 1987 summer field season.

Electromagnetic 60 Hz field strengths for the plots used in small mammal homing were similar for pre-1986 and 1986 periods, and differed by less than a factor of 2 in 1987 (Tables 29-31). Electromagnetic 76 Hz fields ranged from 1 to about 2 fold difference on test and control homing plots in 1986 and 1987 (Tables 34-36).

DEVELOPMENTAL STUDIES

I. Purpose

The purpose of these studies is to determine the incidence of embryonic developmental abnormalities in tree swallows at test and control sites and to test for possible effects of the ELF Communication System on the incidence of these abnormalities.

II. Methods

Embryos of tree swallows are collected from test and control plots in late May and early June. Our procedure is to examine nests daily and mark new eggs. When no further eggs are laid in a nest, incubation is considered to have started. Eggs are then collected from the nest at age 96 hours (4 days) of incubation. Each embryo is dissected from the egg and placed in a fixative (Bouins solution). An initial determination of whether the embryo is normal or deformed is made at the time of dissection. At this time a determination of whether the egg was ever fertilized is made. These eggs are identified by their lack of any embryonic tissues.

The preserved specimens are later cleared, stained, mounted whole

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

on glass slides and examined in detail for a final determination of whether they are normal or abnormal. This final determination is carried out according to a "blind" procedure. All specimens from both test and control sites are assigned arbitrary and randomly selected numbers. The person who carries out the final examination of the embryos knows only these numbers, not the origin of each specimen. Abnormal embryos are categorized according to the particular type of abnormality they show. All embryos are photographed to maintain a permanent record of normal and abnormal embryonic morphology.

III. Results: 1985 to 1987

Embryos were collected from control plots (TMC and PPC), and test plots (PRT, CHT, FST, and FNT -- see Table 1 for plot designations). Because of various problems, embryos were not collected from the same sites in all years. In future seasons, beginning with 1987, embryos will be collected from the same test and control plots as in 1986 to eliminate possible differences between plots within the same experimental treatment group.

The Chi-square analysis of embryos collected in 1985 to 1987 from control plots (Table 24a) illustrates that the plots are not homogeneous with respect to developmental abnormalities observed. This is because the Tachycineta Control Plot (TMC) for 1987 had an unusually high level of developmental abnormalities. If we remove the 1987 TMC data from the analysis, the control data become homogeneous.

The Chi-square analysis of embryos collected in 1985 to 1987 from test plots (Table 24b) indicates that the plots are not homogeneous due to higher abnormalities at the FNT plot, a finding the same as for 1986. If we eliminate the data from FNT, the data from the test plots

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

are homogeneous.

Pooling test and control embryonic data (without TMC or FNT, Table 25), we observed that frequencies of developmental abnormalities were homogeneous with an average of about 9.7%. Examination of FNT plot by itself for 1986 and 1987 indicate the level of abnormalities were homogeneous, giving an average level of developmental abnormalities of 37.0%, more than three times as high as other plots.

Data from the TMC plot from 1986 and 1987 were not homogeneous. Developmental abnormalities were 6.5% in 1986 and 23.5% in 1987 (Table 26). Of the three different levels of developmental abnormalities found (Table 26), the lowest level occurred on control and test plots, excluding the 1987 TMC and all the data from FNT plot. If we assume that this is the normal level of abnormalities in tree swallows (9.7%), we may compare this level with the levels of abnormalities observed on the 1987 TMC plot and FNT plot. Our comparison indicates that there is a significant difference between the pooled plots (9.7%), the 1987 TMC plot (23.5%) and the FNT plots (37.0%). These differences are significant at least at the $P = .025$ level of significance (Chi-square test).

At the present writing, we consider the frequency of developmental abnormalities for the pooled test and control plots as representative of the base rate of abnormalities that we may expect for these plots. The higher levels of developmental abnormalities for FNT plot in 1986 and 1987, and for TMC in 1987, call for an explanation. However, at the present time, we do not know what is causing the higher rates.

The level of abnormalities for FNT do not seem to be

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

associated with the presence of ELF radiation. The other test plots (CHT and FST) have similar electromagnetic field strengths during the same time period (Table 4b), but do not show the levels of abnormalities. Second, FNT had higher EM field levels in 1987 than in 1986 with no change in the abnormality rate (Table 4b). Finally, the control plots in 1986 were homogeneous, but were not in 1987. EM field strength were virtually the same on both plots (TMC and PPC) in 1986 and 1987 (Table 4b).

Always in the past, plots with very high levels of developmental abnormalities (Floodwood and FNT) have been consistent from year to year. We suggest that the increase in the frequency of abnormalities for TMC in 1987 was caused by response to some adverse climatic condition that only manifested itself on that plot. During the 1987 season, there was a hard freeze during the early egg laying period. Differences in the micro-habitat, especially low areas, may accentuate the cold temperatures for birds having nests boxes there. Prior to the start of incubation, such cold snaps could damage embryos. We know that very cold temperatures early in the development of domestic chicken embryos can result in large increases in the frequency of developmental abnormalities (Asher, per observation). We suspect that similar fluctuations in ambient temperatures may have produced the abnormalities observed with the 1987 TMC plot, but as yet do not have temperature data to corroborate it. One additional piece of information tends to support this hypothesis. The FNT plot, which has consistently produced high numbers of abnormal embryos, is situated along the Ford River and has a very low topography. During cold spells, colder air may settle on this plot. The consistent high

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

incidence of developmental abnormalities at FNT could be the result of a consistent local cold spot. We are currently examining daily temperature data collected by other investigators (aquatic studies group) for FNT.

In future years, we will monitor the ambient and in-nest temperatures in each plot to be used for the collection of embryos during the critical egg-laying period. We will then be able to assess the extent to which each plot is exposed to the same general ambient temperatures during the critical egg-laying period.

STUDIES OF MAXIMUM AEROBIC METABOLISM

I. Purpose

The purpose of these studies is to measure the peak aerobic metabolism of animals during winter at test and control sites and to test for possible effects of the ELF Communication System on peak metabolism. The principal species studied are chickadees and deermice.

II. Methods

Collection and care of birds. To attract chickadees for study, feeding stations are established in December and kept stocked throughout the winter with sunflower seeds. Chickadees are mist netted as needed from these stations. Upon capture, birds are weighed to the nearest 0.1 g using a Pesola spring scale and marked with a colored plastic leg band for individual identification. When released from captivity, they are banded using a standard U.S. Fish and Wildlife Service band for permanent marking. Birds are housed singly in wire

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

mesh cages (28 x 18 x 31 cm). Shelled sunflower seeds and snow or water are available ad libitum. In addition, each morning and late afternoon, meal worms are provided in excess. The cages are kept in a screened outdoor holding facility, which provides natural lighting and temperature conditions.

Collection and care of mammals. Trap shelters are established in late November, prior to any substantial snowfall. The shelters are located along wandering lines situated approximately 75-250 m from the antenna or sham corridor. Habitat is northern hardwoods dominated by maple, basswood, and elm, typical of the area. Each shelter is a plastic waste container placed upside-down on top of the ground layer, with a covered top opening which provides researcher access to the ground layer once snow is present. Mice enter the shelters through the interface between the ground layer and the wall of the shelter. One Leathers live trap is placed in the bottom of the shelter and baited with rolled oats, peanut butter, and sunflower seeds. Polyester batting is provided in the trap for nesting material. Traps are prebaited and left open one month prior to actual trapping to insure that small mammals will include the stations in their subnivean runways. Researcher travel on the sites is by snowshoe along a single trail to minimize disturbance of the subnivean air spaces which are critical to small mammal movements.

Trapping is begun at the start of January and continued intermittently -- according to need for animals -- through March. Work is focused primarily on the deer mouse. Upon capture, individuals are toe-clipped for identification, sexed and weighed to the nearest

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

0.1 g with a Pesola spring scale. Once at the lab, animals are transferred to standard plastic lab cages (29 x 18 x 13 cm) with wire lids and provided with wood shavings, polyester batting, and a diet of sunflower seeds, lab chow, and apple and snow for moisture. Cages are housed in an open outdoor facility which provides natural lighting and temperature conditions.

Laboratory methods. To elicit a peak rate of oxygen consumption, we use a refined version of the helium-oxygen (helox) method first introduced to the study of small-animal physiology by Rosenmann and Morrison (1974). Placing an animal in a helium-oxygen atmosphere at a given ambient temperature greatly increases the individual's rate of heat loss by comparison to the rate in air (mostly nitrogen-oxygen), due to the relatively much higher thermal conductivity of helox. Thus, the animal must produce heat more rapidly in helox than air if it is to maintain a stable body temperature.

Whether the rate of oxygen consumption measured in helox is in fact a true peak metabolic rate depends partly upon the ambient temperature. Identifying the true peak for an individual therefore entails studying the animal at a series of ambient temperatures. Specifically, study at a minimum of three ambient temperatures is required for a definitive determination: there should be a measurement at the temperature that elicits the peak, and also there should be measurements at temperatures higher and lower, demonstrating that the rate of oxygen consumption in helox falls off if the temperature is either raised or lowered from that eliciting the peak. Of course, the temperatures of interest are unknown at the onset of work on an individual. Thus, in principle, many measurements would have to be made on an individual before its

peak would be definitively identified. In practice, experience often permits us to know in advance the temperature at which the peak will occur. Therefore, we often need to test an animal at just three temperatures to establish its peak definitively. The spacing we have used between temperatures is 5 °C. Thus, if we test an animal in helox at three ambient temperatures that are 5 °C apart (e.g. -10, -5, 0 °C) and if the highest measured rate of oxygen consumption occurs at the middle temperature, we conclude that we have identified the animal's peak rate definitively.

Tests are not carried out on the day of capture to reduce any effect of capture stress. To further avoid adverse effects of stress, animals are tested only once on any given day.

Prior to a test animals are weighed to the nearest 0.1 g on an Ohaus triple-beam balance, and their body temperature (T_b) is measured by inserting a copper-constantan thermocouple probe 2-3 cm colonically. Then each animal is placed into a metabolic chamber. Chambers are constructed from new one-half gallon paint cans, with inflow and outflow ports in the lid. The inside surfaces are painted with 3M ECP-2200, for an emissivity of nearly 1.0. A 0.5-inch-mesh hardware cloth floor covered with Dip-It plastic coating is used to elevate the animal above the bottom of the can, thus helping to insure proper airflow around the animal and permitting urine and feces to drop away so as not to wet the animal. The outflow port of each chamber houses a 36-gauge copper-constantan thermocouple to monitor chamber temperature, which is maintained by immersion of the can in a Forma Scientific 2325 water bath using ethanol as antifreeze. All temperature probes are connected

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

to a Leeds and Northrup 250 Series Multipoint recorder which can be read to the nearest 0.1 °C.

Measurements are carried out during daylight hours. Food is provided during measurements. Specifically, apple is provided for the mammals, and shelled sunflower seeds and a mealworm are provided for the chickadees. The metabolism chambers for the birds are equipped with a small light that provides dim illumination; without this light, the chickadees (which are diurnal feeders) would not eat. Our decision to provide food during tests is based on extensive preliminary experimentation and is predicated on the following considerations: (1) Animals in nature are able to feed during the day; the birds are diurnal foragers, and the mammals can feed from caches. (2) In the mice, the variance in results is lower when food is provided than when it is denied. (3) In the birds, there is evidence that fasting during these types of experiments increases the probability of death.

Oxygen consumption is measured using an open-flow system. Briefly, gas (air or helox) is pumped through the metabolic chamber at a measured flow rate, and the reduction in its oxygen content is measured. From these data, the rate of oxygen use of the animal can be calculated. The oxygen content of gases is measured with an Applied Electrochemistry S3A oxygen analyzer and recorded on a Houston Superscribe potentiometric recorder. Gas flow rates are measured with Brooks 1110 rotameters. The rate of oxygen consumption is calculated according to the formulas in Hill (1972a, method B), taking cognizance of the mathematical relationship between gas composition and the output of the S3A analyzer. We have empirically verified that the S3A analyzer reads oxygen levels in helox with the same accuracy as in air.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Animals are provided with air during an initial adjustment period (0.7-1.5 hr) and then switched to helox. Flow rates are 600 ml/min in air and 900 ml/min in helox. The adjustment period in air is terminated once the metabolic rate has remained approximately stable for 15 to 20 minutes. Upon switching to helox, a rapid transition to the new gas is made by purging the metabolic chamber at a rate of 5 liters/min for two minutes. Then the rate of flow is reduced to the 900 ml/min already mentioned. The maximal rate of oxygen consumption under the test conditions is generally achieved within 15-20 minutes after the switch to helox, and animals are rarely exposed to helox for more than 25 minutes. Following the measurement in helox, animals are quickly removed from the metabolic chamber, and a final T_b and weight are recorded.

All thermocouples have been calibrated against thermometers whose calibration is traceable to the National Bureau of Standards. Flowmeters have been calibrated against a Brooks Volumeter also having a NBS-traceable calibration.

The one aspect of the measurement procedure that is open to significant subjective judgment is the determination of the particular time interval over which the maximum oxygen consumption occurred in each experiment. Because of the subjectivity involved in this determination, a "blind" procedure will be used once the Communication System antenna has been turned on and high-resolution comparisons of test and control sites are being carried out. The relevant raw data, as earlier noted, are recorded using a potentiometric recorder. These records are not marked as to the origin of the animals (test or control

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

site) but instead are identified simply by arbitrary, randomly assigned numbers. The final and definitive reading of the records will be carried out by a person who knows only these arbitrary numbers.

III. Results - 1987

In 1987 new computer routines for archiving and analyzing the data on peak metabolic rates were acquired from Johnston Computers. The database for this aspect of our research has grown large, consisting of nearly 1100 records, each containing 43 entries. Our original system for computer entry of the data, proofreading, archiving, and analysis had become bogged down as the database grew, and thus we needed a new, professionally designed system. In 1987, the new programs were checked and debugged to meet our operating needs; all data in the old database systems were transferred into the new database; data from the 1987 winter were entered; and a thorough proofreading of all data from all years was completed. Thus, for the first time we are in a position where all metabolic data are in a single database, the integrity of which has been fully checked, and therefore we are prepared to carry out a definitive and final analysis of all the data in 1988.

Definitive peak metabolic rates were obtained for 17 freshly-caught chickadees and 11 deermice in 1987 (low population sizes of deermice continue to interfere with acquisition of animals). Table 27 presents a summary of the data. For each species, we analyzed the 1986 and 1987 data together in a 2 X 2 analysis-of-variance design, with one factor being year and the other being plot (test versus control). In the analysis of weight-specific rates of oxygen consumption, no

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

significant differences were found for deermice between years ($F = 3.2$, $P > .08$) or plots ($F = 1.4$, $P > .25$) or for chickadees between years ($F = 1.5$, $P > .22$) or plots ($F = 1.9$, $P > .18$) [variances were homogeneous for each species according to the Fmax test; interaction terms were nonsignificant ($F = .1$, $P > .75$ for deermice, $F = 2.1$, $P > .15$ for chickadees)]. Body weights also proved to show no significant differences between years or plots for either species. In the analysis of whole-body rates of oxygen consumption (oxygen consumption per animal*hour), body weight was entered as a covariate. The (linear) relation between whole-body rate of oxygen consumption and body weight proved to be highly significant for both deermice ($F = 24.3$, $P < .0001$) and chickadees ($F = 10.0$, $P < .004$), showing that the use of body weight as a covariate reduces variance extraneous to the comparisons of direct interest to us between years and plots. For deermice, no difference was found between plots in the whole-body rates of oxygen consumption ($F = .67$, $P > .42$), but a significant difference between years was found ($F = 6.38$, $P < .02$); for chickadees, no differences between either plots ($F = 2.43$, $P > .13$) or years ($F = 2.2$, $P > .15$) were found [variances were homogeneous according to the Fmax test; interaction terms were nonsignificant for both deermice ($P > .95$) and chickadees ($P > .2$)]. For deermice, the finding of a significant difference between years in the whole-body rate of oxygen consumption with the effects of weight removed (by covariate analysis) is at odds with the finding of no difference in the analysis of weight-specific rate of oxygen consumption; this is enigmatic. However, both the analysis of whole-body rates and that of weight-specific rates agree in both species that there are no differences between test and control

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

plots. This is the finding of greatest import because it is critical to our experimental design that the test and control plots not differ prior to activation of the ELF Communication System.

Table 28 pools data for weight-specific rates of oxygen consumption across both years and plots to provide our best possible estimates of the mean and variance in each species prior to activation of the ELF Communication System. The pooled values differ little from those reported in the annual report for 1986, indicating that our prior estimates of requisite sample sizes to meet experimental objectives remain valid.

We emphasize that the data presented here are definitive peak metabolic rates. By this we mean that each animal was tested at three temperatures with the peak occurring at the middle of the three; recognizing that the temperatures are spaced about 5°C apart, we know for these animals that the measured metabolic rate went down whether we lowered or raised the test temperature by 5°C from the temperature eliciting the reported peak. For many additional animals, we have measures of peak metabolic rate that are not so definitive. For example, in many cases, as we have lowered the test temperature in successive 5°C increments, we have reached a point where a drop in temperature of 5°C caused only a very small rise in measured metabolic rate, indicating that a peak had been reached. Often, in such instances, we have not wanted to lower the temperature still further (to establish the peak definitively) because of the risk of freezing damage or death to animals already near their metabolic limit. We now have a sufficiently large database of definitive peaks and

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

nondefinitive peaks for chickadees and deermice that we will be able to evaluate how closely various sorts of nondefinitive peaks can be expected to approximate definitive peaks. When this analysis is complete, we expect to find that many nondefinitive peaks can reliably be assumed to approximate true peaks, and such nondefinitive peaks will then be incorporated into our peak database (increasing sample sizes markedly).

Studies of potential changes in peak metabolic rate over the course of 2-3 weeks in captivity were completed on 10 deermice and 16 chickadees in 1987. A full analysis of the data of this sort from all years will be carried out with the new database in 1988. Preliminary analysis shows that, as in prior years, the data for 1987 indicate no significant drop in peak metabolic rate over the lengths of captivity of interest.

As has been noted in several monthly reports, we have been working on identifying the causes of some unexplained phenomena in the metabolic apparatus. When a switch is made from air to helium-oxygen (or vice versa), unusual fast transients occur in the oxygen reading of the S3A analyzer, and then there is a slow approach to the true reading for the new gas. These phenomena do not occur when a switch is made from one concentration to another of oxygen in nitrogen or from one concentration to another of oxygen in helium. To determine whether our S3A was at fault, we obtained in 1987 a new sensor unit on loan from the manufacturer, Ameritech. Our finding was that the new unit behaved just like ours, indicating no defects in our unit. In 1987 we also did extensive testing of the metabolic system in numerous configurations designed to elucidate its operating characteristics, and we explored

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

the questions we have with two experts in gas analysis, Dr. John W. H. Dacey of Woods Hole Oceanographic Institution and Dr. Joseph Weissbart, inventor of the S3A analyzer. At this writing the phenomena remain enigmatic to all concerned. For us, the fast transients are of merely academic interest; they are over within 2 minutes, and yet we take no readings for 8-10 minutes following a switch to a new gas. The protracted approach to the true reading of a new gas is a source of error in our measurements, however. Our testing led us to believe that the differential diffusion rates of nitrogen and helium through PVC (polyvinyl chloride) tubing were the most likely cause (our system was extensively plumbed with PVC tubing). Thus, we just recently replaced most tubing with stainless steel. As of this writing, it seems likely from preliminary tests that the problem has not disappeared. Thus, the errors involved may be unavoidable. Peak metabolic rates may be in error by up to 2-3% because of these problems. Although removal of all sources of error has been our goal, errors of this magnitude must be viewed as tolerable, especially in view of the fact that the metabolic rate of an animal often itself varies by 10% or more from the start to the end of our 10-minute measurement periods (also, the errors affect results for test and control animals equally). We shall continue to seek the cause of the slow drift in our system while at the same time being convinced that it does not seriously compromise our results.

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APPENDIX A - TABLES AND FIGURES

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 1. Test-control plot pairings for the various work elements for small mammals and nesting birds. Plot code designations are those used by IITRI.

TEST PLOTS	CONTROL PLOTS	WORK ELEMENTS CARRIED OUT
PIRLOT ROAD (1T1)	MICHIGAMME NORTH (1C1) MICHIGAMME SOUTH (backup only) (1C3)	Small mammal enclosure studies; Small mammal community studies; Small mammal homing studies
PIRLOT ROAD (1T1)	TACHYCINETA MEADOW (1C6)	Tree swallow parental care & growth studies (on plot areas separate from other activities)
CLEVELAND HOMESTEAD (1T2)	PANOLA PLAINS (1C4)	Tree swallow embryology & homing studies
CLEVELAND HOMESTEAD DISPLACEMENT (1D1)	-	Release site for tree swallow homing studies
CLEVELAND HOMESTEAD (1T2)	TACHYCINETA MEADOW (1C6)	Tree swallow embryology
NORTH TURNER ROAD (1T4)	PANOLA PLAINS (1C4)	Tree swallow homing studies.
NORTH TURNER DISPLACEMENT (1D2)	-	Release site for tree swallow homing studies
FORD RIVER NORTH (1T5)	PANOLA PLAINS (1C4)	Tree swallow embryology studies.
FORD RIVER SOUTH (1T6)	PANOLA PLAINS (1C4)	Tree swallow embryology studies.
-	PANOLA PLAINS DISPLACEMENT (1D3)	Release site for tree swallow homing studies
PIRLOT ROAD (1T1)	MICHIGAMME SOUTH (1C3)	Small mammal physiology trapping
PIRLOT ROAD (1T1)	MICHIGAMME NORTH (1C1)	Chickadee physiology trapping

Note: Cleveland Homestead, Ford River North and South plots are small, therefore they have been designated as tree swallow embryology study sites.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 2. Minimum sample size requirements estimated for various study elements to meet the statistical standard of 90% certainty of detecting a 20% change at the 5% level of significance. The procedure follows Sokal and Rohlf (1981, pg 247) for parametric statistics and Gill (1978, pg 82) for frequencies.

STUDY ELEMENT	SPECIES	VARIABLE	ESTIMATED N / PLOT
Parental care, fecundity, growth, and maturation	deermice	litter size	11 females
		weight	21 individuals
		age eye open	6 individuals
		homing likelihood	59 individuals ^{ab}
		% time in nest	35 females ^b
	chipmunk	homing likelihood	44 individuals ^a
	tree swallow	clutch size	23 nests
		egg weight	17 eggs
		likelihood to hatch	44 eggs
		mean hatch rate	52 eggs ^a
		growth rate ^c :	
		weight	58 nestlings
		tarsus	38 nestlings
		ulna	27 nestlings
		wing	6 nestlings
		maturation landmark:	
		feather eruption	7 nestlings
		age eye open	37 nestlings
		fledging rate	337 nestlings ^b
		likelihood to fledge	58 fledglings ^a
		time to fledge	14 fledglings
		homing times	67 birds ^b
		likelihood to home	46 birds ^a
		% time incubating	10 nests
		N nest visits/hour	18 nests ^b
Developmental Abnormalities	tree swallow	frequency of normal embryos	48 embryos ^a
Physiology	deermice	peak metabolism	6 individuals
	black-capped chickadee	peak metabolism	5 individuals

^a Estimated using contingency table procedure in Gill (1978).

^b We consider these sample sizes unobtainable in a single year. However, we expect to be able to pool data across years and thereby meet the established standards.

^c We are currently re-assessing sample needs based on nests, rather than number of individuals.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 3. Summary of mammal community variables at test (Pirilot Road) and control (Michigamme) sites, 1987.

	TEST	CONTROL
DIVERSITY MEASURES^a		
Number of unique individuals (N)	117	110
Total Species Richness (S)	12	10
N used to calculate H'	115	106
S used to calculate H'	10	8
Diversity : H'	1.129	1.598
(Variance)	(0.0163)	(0.0074)
Statistics :	t = 3.045 (P < 0.005)	
	df = 199 (2-tailed)	
Evenness : E (max = 1.00)	.486	.768

RANK CORRELATION OF GENERAL ACTIVITY^b

Spearman's	r = 0.727 (P < 0.02)
	df = 7
Linear Regression	Rank (Control) = 2.036 + 0.555 (Test)
	test of slope of regression; t=2.174 (P=0.073)
	df = 7

^a H' = Shannon-Wiener diversity calculated using Pielou's (1975) method; Variance calculated following Hutcheson (1970).

^b Ranks = number of stations with this species; total of 14 species.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 4a. Estimates of trappable population number (TPN) of chipmunks and deermice at test (Piriot Road) and control (Michigamme) sites for years 1987, 1986 and 1985.^a

Species	Year	TEST		CONTROL		t_s	t_i
		TPN	SLOPE	TPN	SLOPE		
chipmunks	1987	1.00	1.000	10.36	0.815	0.696 P>0.20	6.338 P<0.001
	1986	29.96	4.483	47.56	8.399	1.405 P>0.10	5.919 P<0.001
	1985	58.82	7.244	88.76	14.386	1.913 P>0.05	3.680 P<0.002
deermice	1987	77.83	8.623	40.57	4.187	1.192 P>0.20	6.344 P<0.001
	1986	115.34	19.694	112.38	18.290	0.173 P>0.50	0.152 P>0.50
	1985	145.73	29.311	127.66	18.632	1.257 P>0.20	1.465 P>0.10

^a TPN was estimated using the Leslie method where $TPN = \text{intercept of the curve described by } CI = b_0 + b_1 (NI .5)$, CI = the cumulative number of individuals captured to date, and NI = number of new individuals captured each day ($N = 14$ days; see text). Estimates of the intercept of this relationship were taken from the results of linear regression analyses of the transformed data (i.e., CI as a function of $NI.5$). Slopes and intercepts were tested between plots, t_s and t_i respectively.

Table 4b. Electromagnetic field strengths measured by IITRI from 1983 to the present. Data from 1983 through 1985 are pre-antenna operation, 1986 and 1987 represent initial testing at 4 and 6 amperes on variable schedules (see Appendix B). Values for 1987 are from closest antenna leg.

PLOT	-----60 Hz-----						----76 Hz----		
	Pre-1986 (Mean)			1986			1987		
	T	L	M	T	L	M	T	L	M
CONTROLS:									
MGE(1C1)	0.0	0.09	0.0	0.0	0.10	0.0	0.0	0.02	0.0
MGE(1C3)	0.0	0.16	0.0	0.0	0.08	0.0	0.0	0.20	0.0
PPC(1C4)	0.0	0.04	0.0	0.0	0.06	0.0	0.0	0.0	0.0
(1D3)	-	-	-	0.0	0.05	0.0	0.0	0.01	0.0
TMC(1C6)	0.0	0.08	0.0	0.0	0.07	0.0	0.0	0.0	0.0
TESTS:									
CHT(1T2)	0.0	0.20	0.0	0.0	0.07	0.0	0.08	1.71	0.30
(1D1)	-	-	-	2.5	9.60	0.11	0.0	0.28	0.0
FNT(1T5)	0.0	0.26	0.0	0.0	0.08	0.02	0.13	1.96	0.37
FST(1T6)	0.0	0.57	0.0	0.0	0.23	0.02	0.18	5.40	0.40
NTT(1T4)	-	0.18	0.0	0.0	0.09	0.10	0.14	2.16	0.28
(1D2)	-	-	-	0.0	0.47	0.0	0.0	0.44	0.0
PRT(1T1)	0.0	0.12	0.0	0.0	0.07	0.01	0.08	1.09	0.14

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 5. Tree swallow plots, number of boxes, and percent with egg laying activity on test and control sites for 1985, 1986, and 1987. Egg laying activity is defined as at least two eggs laid before abandonment or continuation of nesting.

PLOT NAME	NUMBER OF BOXES	% ACTIVITY		
		1985	1986	1987
CLEVELAND HOMESTEAD TEST	38	58	62	66
FORD NORTH TEST	17	30	47	41
FORD SOUTH TEST	20	25	55	70
NORTH TURNER TEST	47	23	60	70
PIRLLOT ROAD TEST	36	75	72	78
PANOLA PLAINS CONTROL	75	43	77	87
TACHYCINETA MEADOWS CONTROL	75	43	69	79
TOTALS TEST	158	44	61	68
CONTROL	150	43	73	83

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 6. Tree swallow fecundity data for years 1987, 1986 and 1985. Data are from the Pirlot Road test plot and Tachycineta Meadows control plot and excludes any renests which may have occurred.

Variable	Year	TEST			CONTROL		
		n	\bar{X}	SD	n	\bar{X}	SD
Clutch Size*	1987	24	5.0	0.75	55	5.2	0.81
	1986	23	5.3	0.88	48	4.9	1.01
	1985	21	5.4	0.87	19	4.8	0.86
Hatch Rate**	1987	15	4.2	1.32	40	4.2	1.25
	1986	14	5.1	1.54	30	4.4	1.35
	1985	11	4.4	1.12	10	4.3	1.06
Fledge Rate***	1987	14	3.1	1.99	39	3.1	1.85
	1986	14	1.3	2.27	27	1.2	2.00
	1985	10	3.6	0.84	7	2.6	1.90
Test of Frequency of Clutch Size ^a		G	df	P			
	1987	2.6	2	>0.1			
	1986	3.3	4	>0.3			
	1985	5.4	3	>0.1			

* Clutch size is the maximum number of eggs laid in a nest.

** Hatch rate is the number of eggs which hatch of those available to hatch -- not always the maximum number of eggs in the nest due to occasional predation.

*** Fledge rate is the number of young that fledge from the eggs which hatch, and only include those nests which were followed to completion.

^a Categories of clutch size with fewer than 5 nests were not included.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 7. Likelihood to hatch and fledge for tree swallows in 1987, 1986 and 1985. Data are from the Pirlot Road test plot and Tachycineta Meadows control plot. Comparisons were made using the G test of independence (df=5).

*** HATCHING SUCCESS ***

Year	Plot	Hatch	Not Hatch
1987	Test	63	11
	Control	166	32
1986	Test	71	5
	Control	132	25
1985	Test	48	8
	Control	43	5

Overall $G = 5.89$ $P > 0.25$

*** FLEDGING SUCCESS ***

Year	Plot	Fledge	Not Fledge
1987	Test	44	17
	Control	122	39
1986	Test	18	53
	Control	32	86
1985	Test	36	7
	Control	18	13

Overall $G = 115.78$ $P < 0.001$

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 8a. ANOVA for clutch size of tree swallows. Tested are the effects of PLOT (test and control), YEAR (1985, 1986, 1987) and the interaction of PLOT and YEAR.

SOURCE	DF	TYPE III SS	MS	F	P > F
PLOT	1	3.222	3.222	4.20	0.042
YEAR	2	0.016	0.008	0.01	0.99
PLOT*YEAR	2	4.816	2.408	3.14	0.046
ERROR	184	141.177	0.767		

Table 8b. ANOVA for hatch success of tree swallows. Tested are the effects of PLOT (test and control), YEAR (1985, 1986, 1987) and the interaction of PLOT and YEAR.

SOURCE	DF	TYPE III SS	MS	F	P > F
PLOT	1	1.591	1.591	0.94	0.333
YEAR	2	6.586	3.293	1.95	0.147
PLOT*YEAR	2	2.284	1.142	0.68	0.510
ERROR	114	192.274	1.687		

Table 8c. ANOVA for fledging success of tree swallows. Tested are the effects of PLOT (test and control), YEAR (1985, 1986, 1987) and the interaction of PLOT and YEAR.

SOURCE	DF	TYPE III SS	MS	F	P > F
PLOT	1	2.917	2.917	0.80	0.372
YEAR	2	80.032	40.016	11.02	0.0001
PLOT*YEAR	2	3.258	1.629	0.45	0.640
ERROR	105	381.119	3.630		

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 9a. Age in days at landmark events of eye opening and primary feather eruption in 1987 and 1986. Data are from the Pirlot Road test plot and Tachycineta Meadows control plot. Sample sizes are numbers of individual young. Day of hatching is defined as day zero.

Year	Plot	<u>Eye Opening</u>			<u>Primary Eruption</u>		
		n	\bar{X}	SD	n	\bar{X}	SD
1987	Test	44	7.4	1.84	44	8.5	1.13
	Control	66	6.7	1.48	66	8.5	1.40
1986	Test	18	5.1	1.02	18	8.8	1.11
	Control	42	6.0	0.73	42	9.1	1.52

Table A-9b. Nested ANOVA for age of eye opening in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987 and 1986.

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1987	PLOT	1	9.7421	9.742	1.13	0.298
	NEST(PLOT)	24	206.892	8.621	9.12	0.0001
	ERROR	84	79.433	0.946		
1986	PLOT	1	3.806	3.806	2.76	0.123
	NEST(PLOT)	12	16.566	1.380	2.75	0.007
	ERROR	46	23.117	0.502		

Table 9c. Nested ANOVA for primary feather eruption in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987 and 1986.

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1987	PLOT	1	0.038	0.038	0.01	0.938
	NEST(PLOT)	24	147.644	6.152	15.27	0.0001
	ERROR	84	33.833	0.403		
1986	PLOT	1	0.460	0.460	0.07	0.797
	NEST(PLOT)	12	80.071	6.673	8.48	0.0001
	ERROR	46	36.183	0.787		

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 10. Exposure data and frequency of mortality for eggs, nestlings, and nests in 1987 calculated using the Mayfield method (Mayfield 1961, 1975). Data are pooled from all test and control plots. Comparisons between test and control were calculated using G-tests (Sokal and Rohlf 1981).

*** EGG MORTALITY ***

	EGG EXPOSURE DAYS	EGG MORTALITIES	
TEST	6457	216	G = 24.324 P < 0.001
CONTROL	10195	210	

*** NESTLING MORTALITY ***

	NESTLING EXPOSURE DAYS	NESTLING MORTALITIES	
TEST	3049	41	G = 0.779 P > 0.25
CONTROL	7899	87	

*** OVERALL NEST MORTALITY ***

	NEST EXPOSURE DAYS	NEST MORTALITIES	
TEST	2467	46	G = 7.723 P < 0.01
CONTROL	4510	47	

*** INCUBATION PHASE NEST MORTALITY ***

	NEST EXPOSURE DAYS	NEST MORTALITIES	
TEST	1598	38	G = 7.642 P < 0.01
CONTROL	2563	31	

*** NESTLING PHASE NEST MORTALITY ***

	NEST EXPOSURE DAYS	NEST MORTALITIES	
TEST	867	8	G = 0.068 P > 0.75
CONTROL	1947	16	

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 11a. Nested ANOVA for weight increase in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987, 1986 and 1985 (logistic model used for growth constant).

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1987	PLOT	1	0.00004	0.00004	0.00	0.968
	NEST(PLOT)	24	0.565	0.024	4.23	0.0001
	ERROR	80	0.445	0.006		
1986	PLOT	1	0.0009	0.0009	0.13	0.724
	NEST(PLOT)	12	0.090	0.007	2.43	0.015
	ERROR	46	0.141	0.003		
1985	PLOT	1	0.000009	0.000009	0.00	0.987
	NEST(PLOT)	23	0.722	0.031	8.37	0.0001
	ERROR	80	0.300	0.004		

Table 11b. Nested ANOVA for the inflection point of weight increase in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987, 1986 and 1985 (logistic model used for growth constant).

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1987	PLOT	1	1.563	1.563	0.67	0.423
	NEST(PLOT)	24	56.394	2.350	5.51	0.0001
	ERROR	80	34.094	0.426		
1986	PLOT	1	0.004	0.004	0.00	0.955
	NEST(PLOT)	12	15.241	1.270	3.80	0.0005
	ERROR	46	15.390	0.335		
1985	PLOT	1	3.190	3.190	1.62	0.216
	NEST(PLOT)	23	45.226	1.966	7.12	0.0001
	ERROR	80	22.080	0.276		

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 12a. Nested ANOVA for tarsus growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987, 1986 and 1985 (logistic model used for growth constant).

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1987	PLOT	1	0.001	0.001	0.10	0.753
	NEST(PLOT)	24	0.254	0.011	2.94	0.0002
	ERROR	74	0.266	0.004		
1986	PLOT	1	0.0315	0.0315	2.96	0.111
	NEST(PLOT)	12	0.128	0.0106	4.48	0.0001
	ERROR	44	0.105	0.002		
1985	PLOT	1	0.014	0.014	0.43	0.518
	NEST(PLOT)	23	0.722	0.031	6.50	0.0001
	ERROR	80	0.386	0.005		

Table 12b. Nested ANOVA for the inflection point of tarsus growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987, 1986 and 1985 (logistic model used for growth constant).

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1987	PLOT	1	0.645	0.645	0.10	0.751
	NEST(PLOT)	24	149.639	6.235	5.12	0.0001
	ERROR	74	90.149	1.218		
1986	PLOT	1	1.917	1.917	0.65	0.437
	NEST(PLOT)	12	35.615	2.968	3.80	0.0006
	ERROR	44	34.399	0.782		
1985	PLOT	1	0.538	0.538	0.21	0.648
	NEST(PLOT)	23	57.860	2.516	4.22	0.0001
	ERROR	80	47.662	0.596		

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 13a. Nested ANOVA for ulna growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987, 1986 and 1985 (logistic model used for growth constant).

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1987	PLOT	1	0.0006	0.0006	0.17	0.683
	NEST(PLOT)	24	0.081	0.003	1.29	0.197
	ERROR	80	0.208	0.003		
1986	PLOT	1	0.00007	0.00007	0.02	0.891
	NEST(PLOT)	12	0.0443	0.004	1.21	0.306
	ERROR	45	0.137	0.003		
1985	PLOT	1	0.020	0.020	0.56	0.462
	NEST(PLOT)	23	0.817	0.036	10.76	0.0001
	ERROR	80	0.264	0.003		

Table 13b. Nested ANOVA for the inflection point of ulna growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987, 1986 and 1985 (logistic model used for growth constant).

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1987	PLOT	1	3.213	3.213	1.76	0.197
	NEST(PLOT)	24	43.860	1.828	6.13	0.0001
	ERROR	80	23.841	0.298		
1986	PLOT	1	0.062	0.062	0.08	0.783
	NEST(PLOT)	12	9.281	0.773	5.12	0.0001
	ERROR	45	6.794	0.151		
1985	PLOT	1	6.516	6.516	1.61	0.217
	NEST(PLOT)	23	92.909	4.040	14.11	0.0001
	ERROR	80	22.909	0.286		

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 14. Nested ANOVA for wing growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987, 1986 and 1985 (exponential model used for growth constant).

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1987	PLOT	1	0.00001	0.00001	0.04	0.851
	NEST(PLOT)	24	0.009	0.0004	4.81	0.0001
	ERROR	79	0.006	0.00008		
1986	PLOT	1	0.0003	0.0003	0.54	0.477
	NEST(PLOT)	12	0.007	0.0006	10.56	0.0001
	ERROR	45	0.002	0.00006		
1985	PLOT	1	0.002	0.002	1.60	0.218
	NEST(PLOT)	23	0.032	0.001	12.76	0.0001
	ERROR	80	0.009	0.0001		

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table A-15a. Tree swallow growth constants derived from fitted growth curves. Data are from test (Pirilot Road) and control (Tachycineta Meadows) sites for years 1987, 1986 and 1985.^a

Variable	Year	TEST				CONTROL			
		N	\bar{X}	SD	CV	N	\bar{X}	SD	CV
Weight	1987	44	0.40	0.10	25.18	62	0.39	0.10	24.54
	1986	19	0.42	0.06	15.05	41	0.42	0.06	15.15
	1985	80	0.44	0.11	24.47	29	0.44	0.08	18.84
Tarsus	1987	41	0.25	0.06	24.83	59	0.24	0.08	32.66
	1986	18	0.22	0.05	25.14	40	0.29	0.07	23.68
	1985	79	0.35	0.12	33.67	29	0.31	0.07	22.69
Ulna	1987	44	0.35	0.05	14.82	62	0.34	0.05	15.32
	1986	18	0.39	0.06	16.71	41	0.39	0.05	13.38
	1985	79	0.34	0.11	32.97	29	0.36	0.10	22.71
Wing	1987	43	0.16	0.01	7.78	62	0.16	0.01	7.22
	1986	18	0.16	0.01	5.92	41	0.17	0.01	8.28
	1985	80	0.18	0.02	12.45	29	0.17	0.01	7.48

^a The numbers in this table are from completely reanalysed data and may not agree with figures in earlier annual reports.

Table 15b. Tree swallow inflection points derived from fitted growth curves. Data are from test (Pirilot Road) and control (Tachycineta Meadows) sites for years 1987, 1986 and 1985.^b

Variable	Year	TEST				CONTROL			
		N	\bar{X}	SD	CV	N	\bar{X}	SD	CV
Weight	1987	44	5.83	0.89	15.21	62	5.58	0.96	17.27
	1986	19	6.08	0.75	12.30	41	6.15	0.72	11.66
	1985	80	5.20	0.80	15.40	29	5.79	1.02	17.55
Tarsus	1987	41	1.51	1.69	111.78	59	1.33	1.47	110.96
	1986	18	1.01	1.09	107.83	40	1.73	1.13	65.08
	1985	79	1.95	3.50	179.63	29	1.78	1.31	73.79
Ulna	1987	44	4.94	0.89	18.09	62	4.61	0.74	16.06
	1986	18	5.25	0.46	8.831	41	5.19	0.56	10.74
	1985	79	4.86	1.14	23.52	29	5.66	1.01	17.87
Wing ^a									

^a Inflection point not applicable to curves for wing growth.

^b The numbers in this table are from completely reanalysed data and may not agree with figures in earlier annual reports.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 16a. Minimum detectable differences and power for tree swallow growth constants derived from fitted growth curves for years 1987, 1986 and 1985.

Variable	Year	Actual Detectable		Power	Power w/20% Difference
		N	Difference(%)		
Weight	1987	25	10.9	.30	.42
	1986	13	7.5	.30	.68
	1985	24	11.6	<.30	.36
Tarsus	1987	25	11.0	.30	<.30
	1986	13	19.6	.30	.35
	1985	24	11.0	<.30	.30
Ulna	1987	25	4.3	.30	.99
	1986	13	6.1	.30	.89
	1985	24	10.0	<.30	.30
Wing	1987	25	3.6	.30	1.00
	1986	13	4.0	<.30	.94
	1985	24	4.6	<.30	.90

Table 16b. Minimum detectable differences and power for tree swallow inflection points derived from fitted growth curves for years 1987, 1986 and 1985.

Variable	Year	Actual Detectable		Power	Power w/20% Difference
		N	Difference(%)		
Weight	1987	25	4.3	<.30	.75
	1986	13	7.2	.30	.79
	1985	24	5.5	<.30	.79
Tarsus	1987	25	44.3	.30	<.30
	1986	13	23.2	<.30	<.30
	1985	24	20.8	<.30	<.30
Ulna	1987	25	6.7	.30	.70
	1986	13	6.3	.30	.83
	1985	24	8.0	<.30	.48

Wing ^a

^a Inflection point not applicable to curves for wing growth.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 17a. Nested ANOVA for incubation egg temperature of tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1987.

Source	DF	Type III SS	MS	F	P
PLOT	1	4.091	4.091	0.28	0.5988
NEST(PLOT)	17	394.166	23.186	1.57	0.0720
ERROR	241	3551.910	14.738		

Table A-17b. Analysis of Covariance of mean egg temperature (°C) for control and test birds using mean ambient temperature as a covariate.

Source	DF	Type III SS	MS	F	P
PLOT	1	0.069	0.069	0.01	0.9352
NEST(PLOT)	17	317.691	18.688	1.79	0.0295
AMBIENT	1	1051.521	1051.521	100.93	0.0001
MEAN					
ERROR	240	2500.389	10.418		

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 18a. Statistics for growth rates for deermice young compared by year and plot.

Year	Control				Test			
	N	\bar{X}	Std D	CV%	N	\bar{X}	Std D	CV%
1986	42	0.25	0.091	36.2	50	0.28	0.085	29.9
1987	47	0.38	0.063	16.4	42	0.31	0.777	25.2

Table 18b. Nested ANOVA of deermice growth rates on test (Pirlot Road) and control (Michigamme) sites for years 1987 and 1986. Tested are the effects of plots ,PLOT, and litters within a plot, MOTHER(PLOT).

Year	Source	DF	Type III SS	MS	F-Value	P > F
1987	PLOT	1	0.0008	0.0008	0.03	0.855
	MOTHER(PLOT)	14	0.338	0.0241	31.39	0.0001
	ERROR	71	0.055	0.0008		
1986	PLOT	1	0.054	0.054	3.21	0.095
	MOTHER(PLOT)	14	0.234	0.017	14.25	0.0001
	ERROR	70	0.082	0.001		

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 19. Minimum detectable differences and power for deermice growth constants for years 1987 and 1986.

Year	N	Actual Detectable Difference(%)	Power	Power w/20% Difference
1987	15	0.0557	<.30	.30
1986	15	0.0702	.32	.35

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 20a. Relevant statistics for age of eye-opening and incisor eruption for deermice reared in enclosures during 1987 and 1986.

Year	Plot	<u>Eye Opening</u>			<u>Incisor Eruption</u>		
		n	\bar{X}	SD	n	\bar{X}	SD
1987	Test	44	15.7	1.87	44	6.1	1.55
	Control	43	16.0	1.10	43	6.4	1.66
1986	Test	28	14.1	2.01	28	5.6	1.29
	Control	48	15.0	1.08	48	6.1	1.49

Table 20b. Nested ANOVA of deermice age of eye opening on test (Pirlot Road) and control (Michigamme) sites for years 1987 and 1986. Tested are the effects of plots, PLOT, and litters within a plot, MOTHER(PLOT).

Year	Source	DF	Type III SS	MS	F-Value	P > F
1987	PLOT	1	3.458	3.458	0.28	0.603
	MOTHER(PLOT)	14	170.739	12.196	28.74	0.0001
	ERROR	71	30.124	0.424		
1986	PLOT	1	9.630	9.630	0.76	0.400
	MOTHER(PLOT)	12	151.674	12.640	61.54	0.0001
	ERROR	62	12.733	0.205		

Table 20c. Nested ANOVA of deermice incisor eruption on test (Pirlot Road) and control (Michigamme) sites for years 1987 and 1986. Tested are the effects of plots, PLOT, and litters within a plot, MOTHER(PLOT).

Year	Source	DF	Type III SS	MS	F-Value	P > F
1987	PLOT	1	5.650	5.650	0.40	0.535
	MOTHER(PLOT)	14	195.684	13.977	42.43	0.0001
	ERROR	71	23.391	0.329		
1986	PLOT	1	1.289	1.289	0.14	0.713
	MOTHER(PLOT)	12	109.279	9.107	13.98	0.0001
	ERROR	62	40.390	0.651		

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 21a. Results of the 1987 and 1986 tree swallow homing study. Data are from Panola Plains control plot and pooled from Cleveland Homestead and North Turner test plots. All times are in minutes from release. Returns are those birds which returned to the nesting area in less than 300 minutes. Likelihood to return was assessed using the Chi-squared statistic.

Year	Plot	Return	Not Return	
1987	Test	36	1	
	Control	25	13	
$\chi^2 = 12.259 \quad P < 0.001$				
1986	Test	26	3	
	Control	24	7	
$\chi^2 = 1.6 \quad P > 0.10$				

Table 21b. Mean return times (minutes) of tree swallows compared with a t-test using pooled standard deviations.

RETURN TIMES				
Year	Plot	\bar{X}	SD	n
1987	Test	155.1	46.2	36
	Control	202.4	55.6	25
$t = 3.6 \quad P < 0.0006$				
1986	Test	149.8	52.6	26
	Control	176.9	67.0	22 ^a
$t = 1.6 \quad P > 0.1234$				

^a Only 22 of 24 returns were used in this analysis because two returns had inaccurate times recorded.

Table 21c. ANOVA of tree swallow return times for PLOT (test and control), YEAR (1987 and 1986) and the interaction of PLOT and YEAR.

Source	DF	Type III SS	MS	F-Value	P > F
PLOT	1	36547.316	36547.316	12.29	0.0007
YEAR	1	6256.308	6256.308	2.10	0.1499
PLOT*YEAR	1	2704.631	2704.631	0.91	0.3424
ERROR	105	312224.767	2973.569		

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 22. Numbers of tree swallows used in homing tests categorized by gender and age.

Year	Plot	Sex		Age ^a	
		Male	Female	SY	ASY
1987	Control	17	21	2	19
	Test	16	21	13	8
1986	Control	14	18	9	9
	Test	15	14	8	6

^a Age categories are: SY - second year, the bird hatched the previous summer; ASY - after second year, the bird is two or more years old.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 23. Results of the small mammal homing studies at Pirlot Road test site and Michigamme control site during the summer of 1987.

Species	Plot	Return	Not Return		
Chipmunks	Test	4	8		
	Control	2	2	$G = 0.3037$	$P > 0.50$
Deermice	Test	16	7		
	Control	6	3	$G = 0.0234$	$P > 0.75$

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 24a. Chi-square analysis of tree swallow embryo data from control plots for 1985, 1986, and 1987.

Year	Plot	Normal	Abnormal	Total
1985	TMC	43	3	46
1986	PPC	49	3	52
1987	TMC*	39	12	51
	PPC	22	5	27
Total		153	23	176
Chi-square with 1987 TMC data				= 9.13 P < 0.025 d.f. = 3
Chi-square without 1987 TMC data				= 4.10 P < 0.100 d.f. = 2

* The plot which caused the chi-square to be significant.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 24b. Chi-square analysis of tree swallow embryo data from test plots for 1985, 1986 and 1987.

Year	Plot	Normal	Abnormal	Total
1985	PRT	42	4	46
	CHT	10	1	11
1986	FNT*	11	7	18
	FST	29	4	33
1987	FNT*	23	13	36
	FST	20	4	24
	CHT	46	4	50
Total		181	37	218
Chi-square with FNT data		=	21.59	P < 0.001 d.f. = 6
Chi-square without FNT data		=	1.59	P < 0.500 d.f. = 4

* The plot which caused the chi-square to be significant.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 25. Chi-square analysis of pooled tree swallow embryo data from sites other than 1987 TMC, 1986 FNT and 1987 FNT.

	Normal	Abnormal	Total
Control	114	11	125
Test	147	17	164
Totals	261	28	289
Chi-square = 0.20 P < 0.500			

Table 26. Frequency of abnormal tree swallow embryos from plots not included in the pooled chi-square analysis compared to the pooled frequency.

Plot	Abnormal (%)	N
Test + Control not including FNT and TMC, 1987 (1985 to 1987)	9.7	289
FNT (1986,1987)	37.0	54
TMC (1987)	23.5	51

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 27. Peak aerobic metabolic rates in deermice and chickadees captured at the Pirlot test plot (PRT) and Michigamme control plot (MGE) during the winter of 1987.

SPECIES STUDIED	MGE	PRT
DEERMICE		
Number studied	6	5
Mean peak weight-specific rate of oxygen consumption (ml O ₂ /g*hr)	19.6	18.6
S.D. of peak weight-specific rate of oxygen consumption	0.7	2.3
Mean peak total rate of oxygen consumption (ml O ₂ /animal*hr)	331.0	341.9
S.D. of peak total rate of oxygen consumption	24.2	29.3
Mean body weight (g)	16.9	18.5
S.D. of body weight	1.0	2.0
CHICKADEES		
Number studied	9	8
Mean peak weight-specific rate of oxygen consumption (ml O ₂ /g*hr)	24.1	24.2
S.D. of peak weight-specific rate of oxygen consumption	1.6	1.7
Mean peak total rate of oxygen consumption (ml O ₂ /animal*hr)	276.7	266.4
S.D. of peak total rate of oxygen consumption	20.8	15.7
Mean body weight (g)	11.5	11.1
S.D. of body weight	0.7	0.5

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 28. Summary of data on mean peak weight-specific rates of aerobic metabolism in 1985, 1986, and 1987. All data for 1985 were gathered on animals from test sites. The data listed for 1986 and 1987 are pooled across test and control sites.

VARIABLE	1985	1986	1987	All years
DEERMICE				
Number studied	7	18	11	36
Mean peak weight-specific rate of oxygen consumption (ml O ₂ /g*hr)	20.7	20.3	19.1	20.0
S.D. of peak weight-specific rate of oxygen consumption	2.4	1.6	1.5*	1.7
CHICKADEES				
Number studied	10	17	17	44
Mean peak weight-specific rate of oxygen consumption (ml O ₂ /g*hr)	24.2	24.9	24.1	24.4
S.D. of peak weight-specific rate of oxygen consumption	1.7	2.0	1.6	1.7

*There is a bit of doubt about the appropriateness of pooling the standard deviations for deermice on test and control plots in 1987. Although variances tested homogeneous in the Fmax analysis carried out in connection with our analysis of variance (see text), the two variances for deermice on test and control plots in 1987 -- when tested as a pair -- test to be heterogeneous. Ultimately this will have to be sorted out, but for the moment we defer further analysis to the time when any additional metabolic values have been added to the data set and the set is thus complete (see text). The standard deviations for chickadees are fully homogeneous, and the specific identified pair of values is the only potentially heterogeneous set of values for deermice. Thus, all in all, we think the presentation here is appropriate for the ends to which it is put, namely assessing intrinsic levels of variation so that sample sizes necessary to meet experimental objectives can be estimated.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 29 . Mean values for 60 Hz transverse electric fields (V/m) on test and control plots for years 1983 to 1987. The values in parentheses are the sample n. Values listed by IITRI as <0.001 are treated as equal to 0.001. Plot 1D3 is the release site for tree swallows used in homing studies on control plots, and plots 1D1 and 1D2 are release sites used for test plots.

PLOT	1983	1984	1985	1986	1987
CONTROLS					
1C1	0.001 (1)	0.001 (1)	0.001 (2)	0.001 (2)	0.001 (2)
1C3	0.001 (2)	0.001 (2)	0.001 (1)	0.001 (2)	0.001 (2)
1C4	-	0.001 (3)	0.001 (4)	0.001 (3)	0.001 (3)
1C6	-	0.001 (1)	0.001 (3)	0.001 (3)	0.001 (3)
Average	0.001 (3)	0.001 (7)	0.001 (10)	0.001 (10)	0.001 (10)
1D3	-	-	-	0.001 (1)	0.001 (1)
TESTS					
1T1	0.001 (1)	0.001 (4)	0.001 (6)	0.001 (14)	0.007 (14)
1T2	0.001 (1)	0.001 (1)	0.001 (1)	0.001 (4)	0.042 (6)
1T4	-	0.001 (1)	0.001 (3)	0.001 (5)	0.020 (10)
1T5	0.001 (1)	0.001 (2)	0.001 (2)	0.001 (6)	0.026 (9)
1T6	0.001 (1)	0.001 (2)	0.001 (1)	0.001 (1)	0.028 (7)
Average	0.001 (4)	0.001 (10)	0.001 (13)	0.001 (30)	0.021 (46)
1D1 & 1D2 (Average)	-	-	-	1.251 (2)	0.001 (2)

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 30. Mean values for 60 Hz longitudinal electric fields (mV/m) on test and control plots for years 1983 to 1987. The values in parentheses are the sample n. Plot 1D3 is the release site for tree swallows used in homing studies on control plots, and plots 1D1 and 1D2 are release sites used for test plots.

PLOT	1983	1984	1985	1986	1987
CONTROLS					
1C1	0.041 (1)	0.146 (1)	0.092 (2)	0.100 (2)	0.114 (2)
1C3	0.115 (2)	0.226 (2)	0.133 (1)	0.080 (2)	0.148 (2)
1C4	-	0.034 (3)	0.044 (4)	0.065 (3)	0.052 (2)
1C6	-	0.072 (1)	0.085 (3)	0.068 (3)	0.089 (3)
Average	0.091 (3)	0.110 (7)	0.075 (10)	0.076 (10)	0.099 (9)
1D3	-	-	-	0.052 (1)	0.156 (1)
TESTS					
1T1	0.090 (1)	0.143 (4)	0.116 (6)	0.070 (14)	0.070 (14)
1T2	0.170 (1)	0.220 (1)	0.197 (1)	0.074 (4)	0.059 (5)
1T4	-	0.181 (1)	0.167 (3)	0.086 (5)	0.076 (10)
1T5	0.230 (1)	0.295 (2)	0.235 (2)	0.079 (6)	0.078 (9)
1T6	0.071 (1)	0.765 (1)	0.870 (1)	0.230 (1)	0.297 (7)
Average	0.140 (4)	0.259 (9)	0.210 (13)	0.080 (30)	0.108 (45)
1D1 & 1D2 (Average)	-	-	-	5.035 (2)	1.280 (2)

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 31. Mean values for 60 Hz magnetic fields (mG) on test and control plots for years 1983 to 1987. The values in parentheses are the sample n. Values listed by IITRI as <0.001 are treated as equal to 0.001. Plot 1D3 is the release site for tree swallows used in homing studies on control plots, and plots 1D1 and 1D2 are release sites used for test plots.

PLOT	1983	1984	1985	1986	1987
CONTROLS					
1C1	0.001 (1)	0.001 (1)	0.001 (2)	0.001 (2)	0.001 (2)
1C3	0.001 (2)	0.003 (2)	0.002 (1)	0.001 (2)	0.001 (2)
1C4	-	0.001 (3)	0.002 (4)	0.001 (3)	0.002 (2)
1C6	-	0.003 (1)	0.003 (3)	0.003 (3)	0.003 (3)
	-----	-----	-----	-----	-----
Average	0.001 (3)	0.002 (7)	0.002 (10)	0.002 (10)	0.002 (9)
1D3	-	-	-	0.003 (1)	0.002 (1)
TESTS					
1T1	0.002 (1)	0.003 (4)	0.003 (6)	0.009 (14)	0.010 (14)
1T2	0.001 (1)	0.001 (1)	0.001 (1)	0.025 (4)	0.018 (5)
1T4	-	0.001 (1)	0.001 (3)	0.012 (5)	0.021 (10)
1T5	0.001 (1)	0.002 (2)	0.001 (2)	0.018 (6)	0.026 (9)
1T6	0.002 (1)	0.001 (1)	0.001 (1)	0.020 (1)	0.033 (7)
	-----	-----	-----	-----	-----
Average	0.002 (4)	0.002 (9)	0.002 (13)	0.014 (30)	0.020 (45)
1D1 & 1D2 (Average)	-	-	-	0.057 (2)	0.080 (2)

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 32. Comparison of mean values for 60 Hz fields on test and control plots averaged over the years 1983 - 1987. Fields are coded as T - transverse fields (V/m), L - longitudinal fields (mV/m) and M - magnetic fields (mG).

Field	Control	Test	Ratio of Larger/smaller	Plot effect	
				F	P
T	0.001	0.005	5.0	0.01	0.91
L	0.090	0.159	1.8	7.31	0.007 *
M	0.002	0.008	4.0	8.37	0.004 *

* Significant F ratio.

Table 33. Comparison of values for 60 Hz fields on test and control plots and the corresponding release plots used for tree swallow homing in 1986 and 1987. Fields are coded as T - transverse fields (V/m), L - longitudinal fields (mV/m) and M - magnetic fields (mG).

Plot	Field:	Ratio of larger/smaller		
		T	L	M

Control and Release plot:

Panola Plains (1C4)
and release plot 1D3: 1986 1.0 1.3 3.0

Panola Plains (1C4)
and release plot 1D3: 1987 1.0 3.0 1.0

Test and Release plot:

Cleveland (1T2) and
release plot 1D1: 1986 2500.0 184.6 4.4

Cleveland (1T2) and
release plot 1D1: 1987 47.0 40.7 8.6

North Turner (1T4) and
release plot 1D2: 1986 1.0 5.9 3.0

North Turner (1T4) and
release plot 1D2: 1987 20.0 2.1 3.5

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 34. Mean values for 76 Hz transverse electric fields (V/m) on test and control plots for years 1986 (4 or 6 amperes) and 1987 (15 amperes). The value in parentheses is the sample N. NS refers to the north-south antenna segment; EW to the averaged east-west segments in 1986 and a single measurement for simultaneous operation of both east-west segments in 1987. All measures on test plots were made on the NS segment, except for displacement plots 1D1 and 1D2, which are located north of the northernmost EW segment. All values reported by IITRI as <0.001 were set to 0.001.

PLOT	TRANSVERSE FIELDS (V/m)			
	1986 (4 or 6 amp)		1987 (15 amps)	
	Antenna NS	EW	Antenna NS	EW
CONTROLS				
1C1	0.001	0.001 (2)	0.001	0.001 (2)
1C3	0.001	0.001 (2)	0.001	0.001 (2)
1C4	0.001	0.001 (4)	0.001	0.001 (3)
1C6	0.001	0.001 (3)	0.001	0.001 (3)
<hr/>				
Average	0.001	0.001 (11)	0.001	0.001 (10)
1D1	0.001	0.001 (1)	0.001	0.001 (1)
TESTS				
1T1	0.078	0.001 (14)	0.264	0.001 (14)
1T2	0.085	0.001 (4)	0.301	0.004 (5)
1T4	0.140	0.001 (5)	0.426	0.001 (10)
1T5	0.237	0.001 (6)	0.790	0.002 (9)
1T6	0.182	0.001 (1)	0.544	0.002 (7)
<hr/>				
Average	0.125	0.001 (30)	0.453	0.002 (45)
1D1 & 1D2 (Average)	0.001	0.002 (2)	0.002	0.010 (2)

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 35. Mean values for 76 Hz longitudinal electric fields on test and control plots for years 1986 (4 or 6 amperes) and 1987 (15 amperes). The value in parentheses is the sample N. NS refers to the north-south antenna segment; EW to the averaged east-west segments in 1986 and a single measurement for simultaneous operation of both east-west segments in 1987. All measures on test plots were made on the NS segment, except for displacement plots 1D1 and 1D2, which are located north of the northernmost EW segment. All values reported by IITRI as <0.001 were set to 0.001.

PLOT	LONGITUDINAL FIELDS (mV/m)			
	1986 (4 or 6 amps)		1987 (15 amps)	
	Antenna		Antenna	
	NS	EW	NS	EW

CONTROLS				
1C1	0.021	0.006 (1)	0.085	0.031 (2)
1C3	0.022	0.008 (1)	0.068	0.029 (2)
1C4	0.001	0.001 (1)	0.003	0.003 (3)
1C6	0.001	0.001 (1)	0.005	0.003 (3)
	-----	-----	-----	-----
Average	0.011	0.004 (4)	0.033	0.014 (10)
1D3	0.008	0.004 (1)	0.053	0.019 (1)
TESTS				
1T1	1.089	0.030 (1)	4.244	0.070 (14)
1T2	1.705	0.128 (14)	7.500	0.728 (5)
1T4	2.162	0.082 (4)	7.390	0.303 (10)
1T5	1.958	0.072 (5)	6.600	0.229 (9)
1T6	5.400	0.122 (6)	18.457	0.184 (7)
	-----	-----	-----	-----
Average	1.668	0.063 (30)	7.987	0.244 (45)
1D1 & 1D2 (Average)	0.068	0.225 (2)	0.320	1.015 (2)

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 36. Mean values for 76 Hz magnetic fields (mG) on test and control plots for years 1986 (4 or 6 amperes) and 1987 (15 amperes). The value in parentheses is the sample N. NS refers to the north-south antenna segment; EW refers to the averaged east-west segments in 1986 and a single measurement for simultaneous operation of both east-west segments in 1987. All measures on test plots were made on the NS segment, except for displacement plots 1D1 and 1D2, which are located north of the northernmost EW segment. All values reported by IITRI as <0.001 were set to 0.001.

PLOT	MAGNETIC FIELDS (mG)			
	1986 (4 or 6 amps)		1987 (15 amps)	
	Antenna NS	EW	Antenna NS	EW

CONTROLS				
1C1	0.001	0.001 (1)	0.001	0.001 (2)
1C3	0.001	0.001 (1)	0.001	0.001 (2)
1C4	0.001	0.001 (1)	0.001	0.001 (3)
1C6	0.001	0.001 (1)	0.001	0.001 (3)
	-----	-----	-----	-----
Average	0.001	0.001 (4)	0.001	0.001 (10)
1D3	0.001	0.001 (1)	0.001	0.001 (1)
TESTS				
1T1	0.143	0.003 (14)	0.530	0.001 (14)
1T2	0.301	0.005 (4)	1.164	0.003 (5)
1T4	0.173	0.003 (4)	1.050	0.002 (10)
1T5	0.337	0.007 (6)	1.409	0.002 (9)
1T6	0.400	0.007 (1)	1.043	0.005 (7)
	-----	-----	-----	-----
Average	0.225	0.004 (29)	0.972	0.003 (45)
1D1 & 1D2 (Average)	0.001	0.002 (2)	0.002	0.010 (2)

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 37. Comparison of mean values for 76 Hz fields on test and control plots averaged over the years 1986 and 1987. Fields are coded as T - transverse fields (V/m), L - longitudinal fields (mV/m) and M - magnetic fields (mG). NS refers north-south antenna segment and EW refers to the two east-west segments. See Table 34-36 for samples sizes for means.

1986

Segment:

NS

EW

Field	Plot:	Test	Control	F	P	Test	Control	F	P
T		0.125	0.001	1.98	0.17	0.001	0.001	-	-
L		1.668	0.011	10.94	0.002	0.063	0.004	8.34	0.007
M		0.225	0.001	2.90	0.099	0.004	0.001	2.62	0.11

1987

Segment:

NS

EW

Field	Plot:	Test	Control	F	P	Test	Control	F	P
T		0.453	0.001	2.17	0.15	0.002	0.001	0.94	0.34
L		7.987	0.033	17.22	0.001	0.244	0.014	12.66	0.001
M		0.972	0.001	8.51	0.005	0.003	0.001	5.23	0.026

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

Table 38. Comparison of values for 76 Hz fields on test and control plots and the corresponding release plots used for tree swallow homing in 1986 and 1987. Fields are coded as T - transverse fields (V/m), L - longitudinal fields (mV/m) and M - magnetic fields (mG). Values are averaged for NS and EW antenna segment operation.

Plot	Field:	Ratio Larger/smaller		
		T	L	M

Control and Release plot:				
Panola Plains (1C4)				
and release plot 1D3:	1986	1.0	6.0	1.0
	1987	1.0	12.0	1.0
Test and Release plots:				
Cleveland (1T2)				
and release plots	: 1986	21.5	6.2	76.5
(1D1 & 1D2)	1987	25.4	6.2	97.3
North Turner (1T4)				
and release plots	: 1986	35.3	7.6	44.0
(1D1 & 1D2)	1987	35.6	11.5	87.7

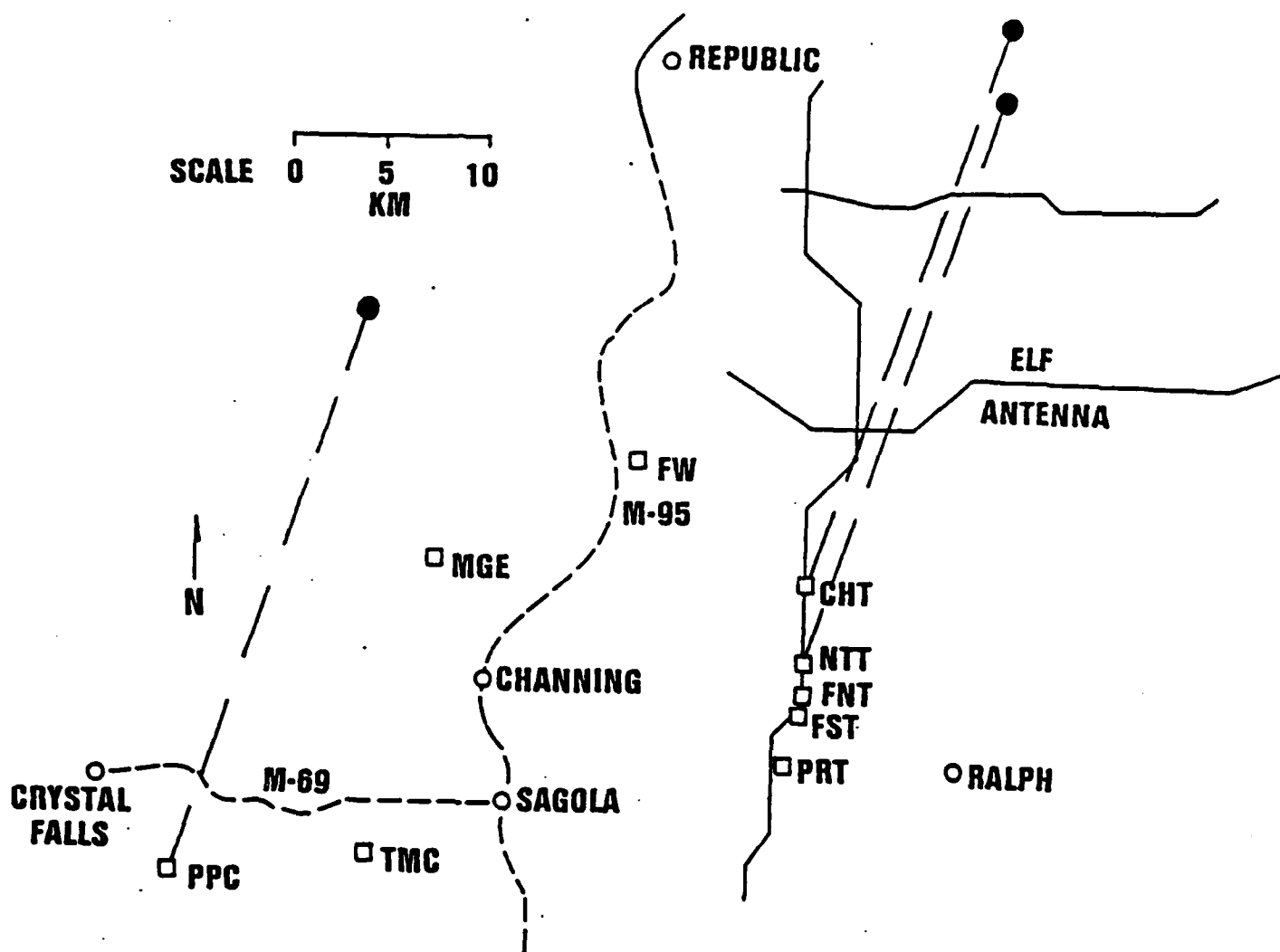


Figure 1. Control and test plots in relation to the ELF Communication System in Michigan. Control plots as referred to in the text are: MGE - Michiganme (North and South parts), PPC - Panola Plains, TMC - Tachycineta Meadows. Test plots are: CHT - Cleveland Homestead, NTT - North Turner; FNT - Ford North, FST - Ford South, PRT - Pirlot Road. FW is Floodwood work plot which was used in the past for tree swallow studies on embryology and homing. Also shown are the release sites used for tree swallow currently.

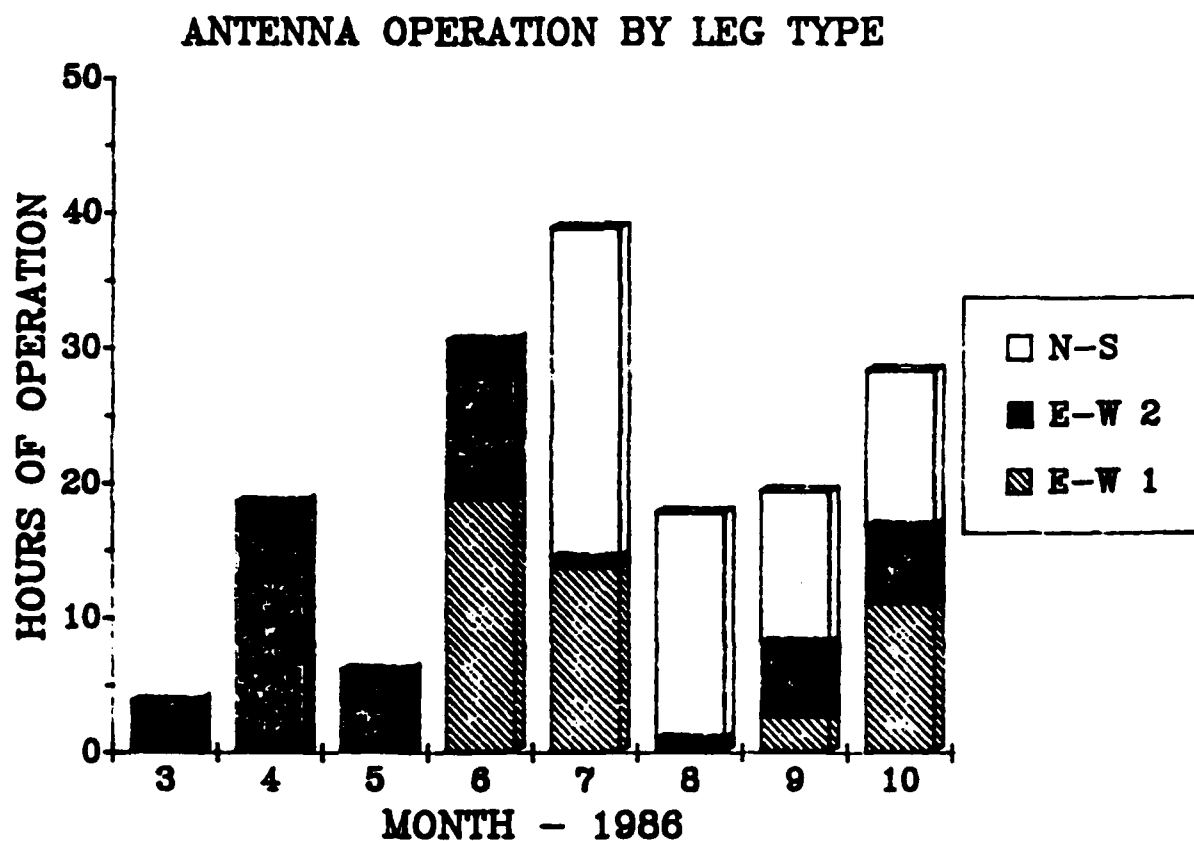


Figure 2. Antenna operation log by antenna leg. Legs are designated as north-south, northern east-west (E-W 1), and southern east-west (E-W 2). Data provided by IITRI.

SMALL MAMMALS AND NESTING BIRDS 1987 ANNUAL REPORT

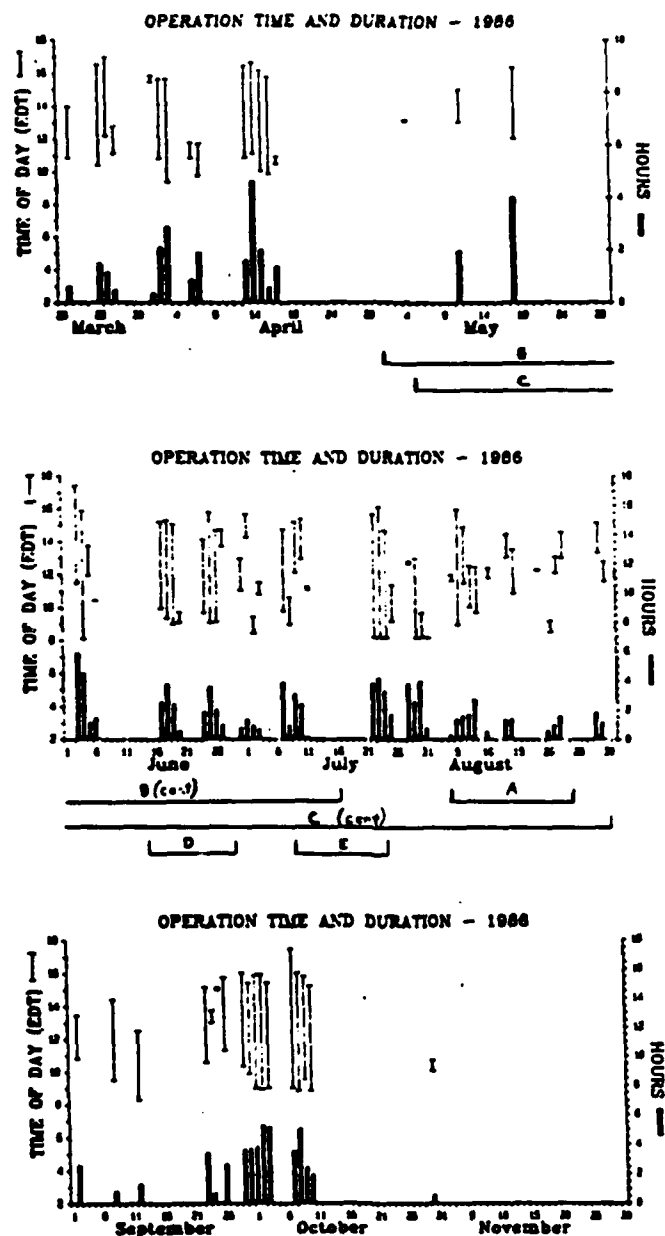


Figure 3. Operation schedule and duration during 1986. Indicated below the date is the research activity in progress. Codes indicate: A - small mammal community studies, B - tree swallow, B - tree swallow nesting, egg-laying, embryology, hatching, growth and fledging studies. C - deer mouse growth and parental care studies, D - tree swallow homing, E - small mammal homing.

OPERATION TIME RANGE - 1987

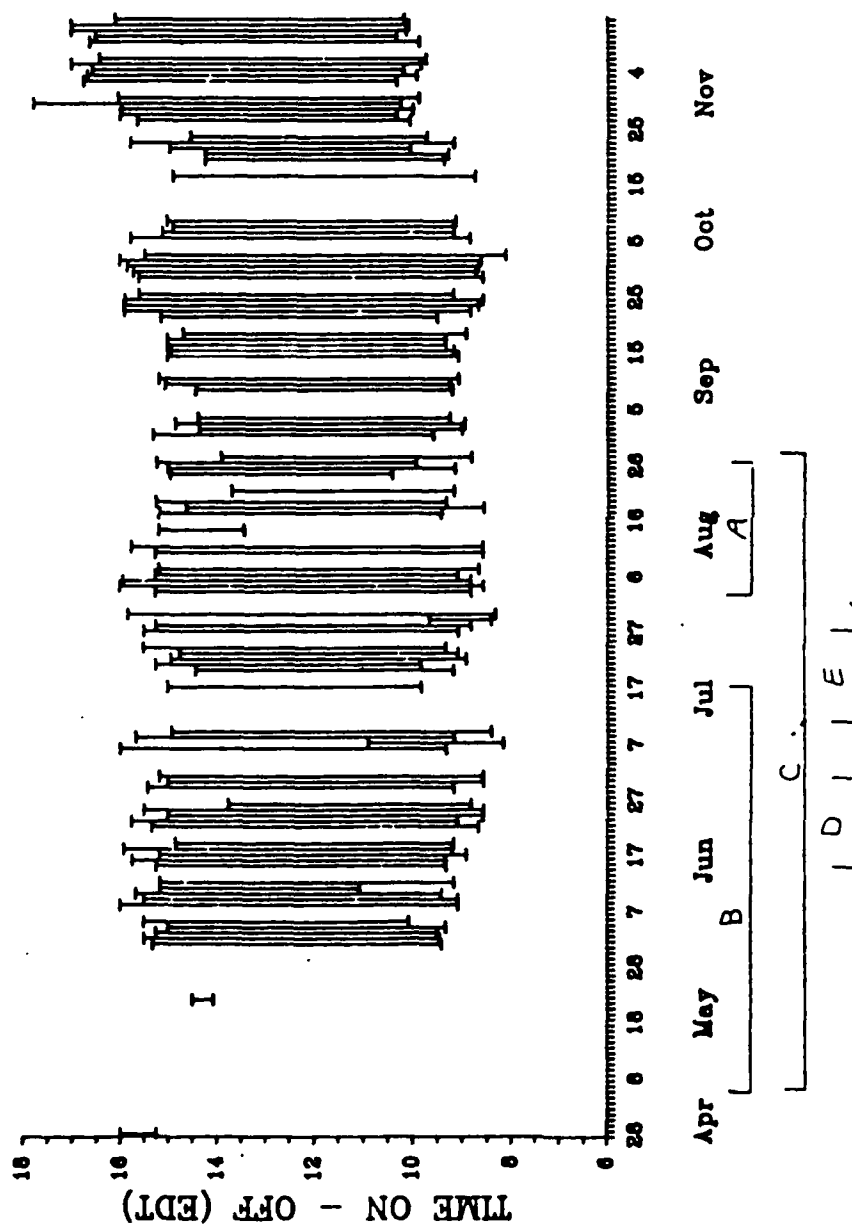


Figure 4. Operation schedule and duration during 1987. Indicated below the date is the research activity in progress. Codes indicate: A - small mammal community studies, B - tree swallow nesting, egg-laying, embryology, hatching, growth and fledging studies. C - deer mouse growth and parental care studies, D - tree swallow homing, E - small mammal homing.